THE

SCIENTIFIC PROCEEDINGS

OF THE

ROYAL DUBLIN SOCIETY.

Vol. XVI. (N.S.), Nos. 30–34. FEBRUARY, 1922.

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DUBLIN:

PUBLISHED BY THE ROYAL DUBLIN SOCIETY,

LEINSTER HOUSE, DUBLIN.

WILLIAMS AND NORGATE,

14, HENRIETTA STREET, COVENT GARDEN, LONDON, W.C. 2.

1922.

Price Seven Shillings.

Royal Dublin Society.

FOUNDED, A.D. 1731. INCORPORATED, 1749.

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XXX.

SOME FACTORS AFFECTING THE HYDROGEN ION CONCENTRATION OF THE SOIL AND ITS RELATION TO PLANT DISTRIBUTION.

By W. R. G. ATKINS, O.B.E., Sc.D., F.I.C.

(Communicated by Professor H. H. Dixon, SC.D., F.R.S.)

[Read November 22, 1921. Published February 2, 1922.]

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Introduction.

It has long been customary to speak of certain soils as acid and of others as alkaline, the terms being used as a rule only when these characteristics were displayed to a marked degree so as to have obvious effects upon the vegetation.

Concerning the nature of soil acidity, a long-standing controversy is still in being, and the views put forward have recently been summarized by

Fisher (1921). Whether the acidity is produced by humic or other organic acids, by the selective absorption of bases, by the interaction of salts with insoluble alumino-silicates, by the presence of alumino-silicic acids, or by the presence of sulphur acids derived from pyrites, there is only one method of stating precisely the effective acidity—namely, by recording the concentration of hydrogen ions in the soil solution or soil extract. This may be done by giving the hydrogen ion concentration in grams per litre, symbolised as C_H or $[H^+]$, or by using the expression pH, which is defined as the logarithm of the reciprocal of the hydrogen ion concentration expressed in grams per litre. For a discussion of the advantages of this method of stating acidity, reference may be made to Clark (1920). But to render what follows more intelligible to those biologists who are familiar neither with the methods by which hydrogen ion concentrations are determined nor with the terminology adopted, a brief account of the subject is given here.

(a) *Hydrogen ion concentration and acidity.*—The acidity of soils has been measured in various ways, of which an account is given by Clark, but it is found that the behaviour of the soil solution or soil extract towards plants is not correctly shown by titration, which gives a measure of the total replaceable hydrogen, not of the concentration of the hydrogen ion effective at any instant. It is by the latter that the effect on the plant is governed, as well as the effect on enzyme action and the solubility of various salts. For example, equal volumes of N/100 hydrochloric and acetic acids require identical amounts of alkali for their neutralization, but the former solution has a far greater concentration of hydrogen ions than has the latter, since in dilute solution hydrochloric acid is almost completely ionized, whereas acetic is not; it is accordingly said to be a weaker acid. If it is assumed that the ionization of hydrochloric acid is complete at this dilution, its concentration in terms of hydrogen ions is C_H or $[H^+] = 1 \times 10^{-2}$ grams per litre. This may be written

$$[H^+] = \frac{1}{10^2}, \text{ or } pH = \log \frac{1}{[H^+]} = -\log [H^+] = 2.$$

It may at first sight appear to be both cumbersome and unnatural to use such an expressson as pH, viz., $-\log [H^+]$, to denote hydrogen ion concentration, but in practice it is extremely convenient, and gives simple numerical values which are easily remembered. Moreover, since an increase of unity in a pH value denotes a decrease to one-tenth in the hydrogen ion concentration, it is obvious that for any graphical presentation of results difficulties arise when the changes are of the order of 10^{-3} or 10^{-6}, yet these are quite common in researches on this subject. For certain purposes, however, it is at

times convenient to use C_H values. To convert the – log $[H^+]$ or pH values into C_H or $[H^+]$ values use may be made of semi-logarithmic paper, as pointed out by Roaf (1920). The first decimal points of the pH values are marked off from right to left as abscissae, the C_H values from 0·1 to 1·0 being ordinates against the logarithmic rulings. A diagonal being drawn, the C_H value of any pH value may be read off, the whole number of the latter being the negative power of ten, by which the C_H value so read must be multiplied. Thus, for example, with pH 4·50 it is found that 0·50 on the abscissa corresponds to 0·32 on the ordinate; the C_H value is then $0{\cdot}32 \times 10^{-4}$. Conversion tables are, however, given by Clark, also at shorter intervals by Schmidt and Hoagland (1919). In making a graph for converting pH into C_H values, it should be noted that the logarithmic scale, as on a slide rule, begins at 1, not 0. A further reason for using – log $[H^+]$ values will be given later on.

(b) *Buffer action.*—If alkali is added to acid, after a certain amount has been run in, a neutral solution is obtained. With a strong acid, such as hydrochloric, the addition of the alkali in successive portions results in a progressive diminution in the hydrogen ion concentration, as may be seen from the fact that the strong acid was largely ionized at the start. But with a weak acid the neutralization of the hydrogen ions existing at any instant results in a new equilibrium being attained by the remaining undissociated molecules of the acid, which become ionized to the same percentage as before. The alteration in the hydrogen ion concentration is therefore much less over a considerable range. If the results are plotted with pH values as ordinates and cubic centimetres of alkali added as abscissae, the slope of the curve will be less steep for the weak acid than for the strong, except near the neutral point. Curves illustrating this are given by Clark (1920) and by Fisher (1921), and in the older literature. A weak acid thus has a considerable "buffer action" in preventing rapid alterations in the pH values, and the same is true of weak bases. Thus peptone and albumen solutions alter in pH value comparatively slowly when acids and alkalis are added. The same is true of many soil suspensions, such as those of peat and calcareous silt; and though these are not solutions, yet they may be considered as having a considerable buffer action, since they yield a continuous supply of a very dilute solution of acid or alkali till exhausted.

(c) *The determination of hydrogen ion concentrations.*—From theoretical considerations Nernst developed an equation connecting the electromotive force of a concentration cell with the concentration of its ions. Platinum black deposited on platinum and immersed in a solution through which pure hydrogen is bubbled constitutes a hydrogen electrode. On the assumption that in very dilute solutions the ions obey the gas laws, it has been shown

that the potential of the hydrogen electrode changes with the concentration of hydrogen ions as follows :—

$$dE = \frac{RT}{nF}\frac{dP}{P},$$

which on integration becomes

$$E = \frac{RT}{nF}\log_e P + A,$$

where A is an integration constant, R the gas constant, T the absolute temperature, n the valency of the ion, and F the faraday or quantity of electricity carried by one gram equivalent of the ion, and $\log_e P$ is the natural logarithm of the partial pressure due to the hydrogen ions. Now, if two such hydrogen electrodes are connected to form a concentration cell, the electromotive force developed is equal to $\frac{RT}{nF}\log_e\frac{C}{C'}$, where C and C' are the concentrations of hydrogen ion, since the ratio of the pressures may be taken as equal to the ratio of the concentrations.

In practice such a hydrogen electrode is connected by an inverted U-tube arrangement, containing a solution of potassium chloride, with a calomel electrode, and the electromotive force of this cell is measured by means of a potentiometer, with all due precautions. The constants being evaluated, use is then made of the following equation to determine the hydrogen ion concentration :—

$$\frac{\text{E.M.F. (obs.)} - \text{E.M.F. (of normal hydrogen and calomel electrode cell)}}{0{\cdot}0001983\ T} = \log\frac{1}{[H^+]} = \text{pH}.$$

Using an N/10 KCl-calomel electrode, this becomes at 25° C.

$$\text{E.M.F. (obs.)} - 0{\cdot}336 = 0{\cdot}9591 \log\frac{1}{[H^+]} = 0{\cdot}0591\ \text{pH}.$$

The fact that the term $\log\frac{1}{[H^+]}$ is thus directly determined is another reason for expressing hydrogen ion concentrations in $-\log[H^+]$ or pH values.

Full proofs of these equations and directions as to the technique may be found in "The Determination of Hydrogen Ions," by Clark.

The method is used as the fundamental one for determining pH values. By its means buffer solutions of accurately reproducible pH values may be standardized for use in colorimetric determinations by means of indicators.

(d) *Neutrality.*—It has not been explained as yet what is meant by a neutral solution or neutrality. Pure water dissociates primarily into hydrogen and hydroxyl ions, and the product of the ionic concentrations is a constant at constant temperature, namely,

$$[H^+] \times [OH^-] = k[H\ O] = K.$$

Since the ions are produced by pure water in equal numbers, the concentration is the same for both when reckoned in gram-equivalents. A solution is accordingly termed neutral when the hydrogen and hydroxyl ions are present in equivalent amounts, as in pure water. With an acid solution the hydrogen ion is in excess, and the hydroxyl is correspondingly reduced, since the product is constant. In alkaline solutions hydroxyl ions preponderate; but as the product is constant, it is possible and convenient to state the reaction of the solution in terms of hydrogen ion concentration rather than in terms of the hydroxyl. One scale, the pH or - log H, is therefore obtained instead of two, based upon the concentrations in pure water, as a starting-point for both.

(e) *The hydrogen ion concentration of pure water.*—It is of interest to determine the hydrogen ion concentration of pure water, but this is attended by many experimental difficulties. Those due to the solution of minute traces of glass may be avoided by the use of silica or platinum vessels, but the absorption of carbon dioxide is still a trouble. It is not possible to determine the pH value by the potentiometer on account of the great internal resistance of the hydrogen electrode half-cell when made up with pure water. The various methods adopted have recently been reviewed by Beans and Oakes (1920). They are as follow:—(1) By deduction from measurements of the electromotive force of concentration cells made up with dilute solutions. (2) By conductivity methods, giving the ionic mobilities of hydrogen and hydroxyl ions in very dilute solutions, which are considered to be valid for pure water; the conductivity of the latter is then measured, and from it and the ionic mobilities the concentration of the ions is calculated. This is the method used by Kohlrausch and Heydweiller (1894), and is the only one of these methods based upon an examination of pure water, but even then use is made of data derived from solutions. For pure water these workers found the hydrogen ion concentration at 26° C. to be $1{\cdot}10 \times 10^{-7}$, or pH 6·96. The value varies with the absolute temperature, as may be seen from the equation given previously. Michaelis (1914) gave for pure water at 16° C., pH 7·10; at 22° C., pH = 7·00; and at 28° C., pH 6·90 (3) By methods based on the hydrolytic dissociation of salts. (4) By

measurements of the rates of certain reactions. (5) By the use of coloured indicators, which give definite tints at known concentrations.

All these methods agree in giving values which are not very far from pH 7·1. Method (5) is used by the writer to test the purity of freshly boiled distilled water and to test glass apparatus for solubility. As shown by its behaviour to brom thymol blue and phenol red, and compared with Clark's standard solutions, the purest, freshly boiled, thrice distilled water which has been prepared here gave pH 7·10–7·05 at about 15° C. Boiling to remove the carbon dioxide in certain types of "hard" glass tube may, however, give any value from about pH 8 upwards, so every tube has to be tested and re-tested after use with alkaline precipitates. If used in the cold, however, such "hard" glass tubes are safe, whereas soft glass tubes are not. Once the indicator has been added the liquid is no longer pure water, hence method (5) is open to objection; but if due precautions are taken in making up the indicator, and if only a very minute amount, say two drops of a 0·02 per cent. solution, is added to 10 c.c. of liquid, the error from this source is slight. Precautions are necessary on account of the fact that pure water has a negligible buffer action.

An entirely new method has been introduced by Beans and Oakes (1920). In this the hydrogen electrode is set up with the purest water, and the cell

$$\mathrm{Hg} \mid \mathrm{HgCl} \,.\, \mathrm{KCl} \mid \mathrm{KCl} \mid \mathrm{H_2O} \mid \mathrm{H_2}$$

is used to charge a condenser of one microfarad capacity, which it does in three to five minutes, whereas a cell of lower internal resistance in which dilute acid is substituted for pure water only requires an instant. Now, the quantity of electricity stored is equal to the product of the E.M.F. of the cell and the capacity of the condenser, viz. $Q = EC$. When discharged through a ballistic galvanometer a deflection d_1 is obtained when the pure water hydrogen electrode is used. Using a standard cell of known voltage a deflection d_2 is shown. Now, since d is proportional to Q, and $Q = EC$, therefore

$$d_1/d_2 = \frac{E_1 C}{E_2 C} \quad \text{or} \quad E_1/E_2 = d_1/d_2 \,;$$

and, since E_2 is known, E_1 may be found, and the pH value calculated as in the potentiometer method. The value arrived at for pure water at 25° C. is pH 7·91, or $[H^+]$ $1{\cdot}23 \times 10^{-8}$. This differs considerably from the results previously obtained, but the method seems very simple and direct. From the biological standpoint it is not of great importance to ascertain this constant precisely, as pure water is never found in nature, and its reaction is

not necessarily the most favourable for living cells, which can, in different species, tolerate a large range of acidity and alkalinity, a reaction suitable for one being too acid for another.

(f) *The colorimetric method of determining hydrogen ion concentrations.*—In addition to the electrical method, using a potentiometer and hydrogen electrode, or the more recent method with a condenser, which requires a less expensive outfit, the colorimetric method introduced by Friedenthal (1904) and Salm (1904) is available. It is based upon the potentiometer method as a standard, the latter being used to determine the pH values of the buffer solutions, made up to be at convenient intervals on the pH scale. Measured amounts of various indicators are added to these solutions. The electrical method is also used to check sources of error, such as those due to proteins and salts, which cause the indicators to give readings higher or lower than the true values.

The colorimetric method was improved and extensively tested by Sörensen (1909), who introduced new indicators, and eliminated those liable to mislead. More brilliant water-soluble indicators of the sulphone phthalein series were introduced by Clark and Lubbs (1917), as well as standard buffer solutions, having certain advantages over those of Sörensen. The indicators and standard solutions used in this research are those described by Clark and Lubs. A full account of the method is also given by Clark (1920) and by Cole (1920). Clark gives a coloured chart, which can also be purchased separately, and is a great aid to field work and to approximate work in the laboratory. In most cases the colours are faithful renderings of those in the standard tubes, with the same indicator in the specified amount.

It may be added that the preparation of standard buffer solutions by each worker is no longer a necessity, as, owing to the work of Meacham, Hopfield, and Acree (1920), a stable series of salts has been found, which is issued on the market in tabloid form. This series covers the range from pH 2·65 to 9·65 by twenty-eight standards, at convenient, but irregular, intervals. Those in the Clark and Lubs series are uniformly pH 0·2, from pH 1·2 to pH 10·0. In work of this nature care should be taken to check and cross-check the solutions and indicators, both when freshly made up and after storing. It is always advisable to use two or more indicators in orienting experiments, and the use of several indicators greatly increases the accuracy of work carried out without standard tubes.

Methods have also been devised for colorimetric determinations without recourse to standard buffer solutions, by employing, in varying number,

drops of indicators in pairs of test tubes. In one set the full acid tint is developed; in the other the full alkaline tint. On looking through the pairs of tubes intermediate shades are seen. The details are given by Barnett and Chapman (1918), Gillespie (1920), Van Alstine (1920), and Medalia (1920).

(g) *Methods of determining soil reaction.*—Numerous measurements of the hydrogen ion concentration of soil extracts have been carried out by the potentiometer method, notably by Fischer (1914), Sharp and Hoagland (1916), Gillespie (1916), Kappen (1916), Plummer (1918), and many recent workers. To establish the applicability of the colorimetric method to soil investigations, Gillespie (1916) carried out a comparison, using the indicators and standard buffer solutions introduced by Clark and Lubs. He found such a reasonably good agreement as to warrant the general use of the colorimetric method. The agreement holds to within pH 0·2–0·4, but the divergence is usually less. Though the electrical method is regarded as the standard, it is for some solutions open to serious objections owing to chemical changes, such as the reduction of nitrates taking place on the platinum black. Difficulties are encountered also in ensuring that the dissolved gases are not swept out by the hydrogen stream; and though these have been surmounted, both for rapidity and delicacy to a degree well within that required for most biological work, the colorimetric method is to be preferred when based upon carefully prepared standards, which should be checked by the electrical method, if possible, for specially accurate work. The determination of the reaction of the soil has found very numerous applications in agriculture and the related sciences; and since, so far as the author is aware, they have not as yet been utilized in Irish agriculture, a short account will be given of a few of these.

Agricultural application of studies on soil reaction.

The methods have been used, for example, in determining the lime requirements of acid soils by Joffe (1920), Blair and Prince (1920), and by Fisher (1921). Now the soil acidity may in itself be injurious if sufficiently great, or it may simply denote deficiency in alkaline calcium salts, to a shortage of which the evil results may in reality be due, or acidity may lead to the production of poisonous aluminium ions, as will be mentioned later.

Again, the reaction of the medium has a pronounced effect upon the growth of bacteria, protozoa, and fungi.

Gillespie and Hurst (1918) found that when grown in soils of acidity pH 5·2 or more acid, potatoes were rarely affected with scab. Thus those

grown on Caribou loam of pH 4·8 were unaffected, whereas in the less acid Washburn loam much damage resulted. They draw attention to the fact that absolute neutrality is not necessarily the most suitable reaction for a crop. Lipman (1919) has accordingly suggested that the reaction of the soil should be altered to suit the crop, this being effected on the acid side by the addition of sulphur, which is oxidized both by air alone and by bacteria in the soil. This condition is favourable for potatoes. By suitable liming it is then possible to neutralize the soil, thus rendering it fit for the leguminous crops of the rotation, such as clover and alfalfa. This is obviously only possible with certain types of soil.

Working on these lines, Martin (1920) has shown by a most convincing quantitative study how the potato scab, *Actinomyces chromogenus*, Gasperini, may be checked on the Irish Cobbler potato. The soil at the start was at pH 6·03, but treatment with 400 and 600 pounds of sulphur per acre altered the acidities to pH 5·20 and 5·07 respectively, the percentage unsaleable through scab decreasing from 42·9 in the untreated soil to 8·9 and 7·5 respectively in the treated, the total crops being closely the same.

It has, moreover, been shown that there is an intimate relation between the growth of nitrifying organisms and soil reaction. Thus Hesselman (1917–1919) found that both nitrate formation and denitrification are stopped by the acid soil in the mossy type of conifer forest in Sweden. Arrhenius (1920) studied the relation between the pH value of the soil and the organically combined nitrogen, nitrate nitrogen, and nitrogen as ammonium compounds. The maximum for nitrate nitrogen was found at about pH 6·8, after which it decreased rapidly, being reduced to one-sixth at pH 5·5. Nitrogen as ammonia increased sharply from pH 6·3 to 5·8, being nearly twice as great at the latter, but after a flat portion the curve falls, the value at pH 4·8 being identical with that at pH 6·3. Throughout these changes the total nitrogen was much the same.

In direct work on the organism, Gainey (1918) proved the extreme sensitiveness of Azotobacter to slight changes in reaction, pH 6·0 being given as its upper limit. Fred and Davenport (1918) ascertained the critical pH values for the root nodule bacteria of various legumes. For example, the bacteria of alfalfa and sweet clover were unable to stand acidity greater than pH 4·9, whereas those of lupines could withstand up to pH 3·15, other types being ranged intermediately. The alkaline limit appears to be the same for all. Growth is, of course, hindered in the neighbourhood of the limiting concentrations.

Furthermore, the oxidation of various carbohydrates is more rapid in alkaline than in acid solution or suspension. Accordingly, quite apart from

biological decomposition, the accumulation of carbohydrates and similar organic matter is favoured by an acid reaction, and in alkaline soils, such as those of Bihar, India, organic matter may become reduced to such low amounts as to lessen crop production (Davis, 1920).

As mentioned further on, changes in soil reaction induced by manuring may be appropriately studied by the indicator method.

The relation of the ions of the soil solution to its hydrogen ion content.

When studying soil reaction it is well to consider the maximum possible effects produced by the ions present, considered salt by salt. Most attention in this country and the eastern states of U.S.A. has been given to soil acidity, but in some western states, in India, Egypt, South Africa, and elsewhere, alkalinity is the bugbear. The question has been discussed in a recent monograph by Harris (1920). In popular language, "alkali soil" denotes both soil sterile from abundance of neutral salts, which is termed "white alkali," and the truly alkaline "black alkali" soil, in which sodium carbonate is present. The action of the latter on the organic matter gives rise to the black colour. White alkali soil may be reclaimed by irrigation with efficient drainage, whereas black alkali soil is far less tractable, since the alkalinity deflocculates the clay particles.

(a) *Calcium salts.*—Consider now the reaction of water in contact with pure calcium carbonate, such as calcite crystals. This may be taken as the first stage in the production of an alkaline reaction. In the—hypothetical in nature—absence of carbonic acid, the salt has a very slight solubility, which, according to Ruppin (1909), quoting Schloesing, is at 16° C. 0·0131 grams per litre, and has a hydroxyl number of $1{\cdot}05 \times 10^{-5}$, namely, that number of gram equivalents per litre. This corresponds to pH 9·015. Accordingly this limit cannot be surpassed in the more alkaline direction by a solution of pure calcium carbonate In order to test this directly, pure calcite crystals were boiled with water both immediately and after standing for some days. As tested colorimetrically by the method of Clark and Lubs, pH 9·0 was only very slightly surpassed by the saturated solution. With limestone, which may contain small amounts of magnesium, pH 9·2 was reached. The standards are at intervals of pH 0·2, but interpolations may be made. With clear solutions and indicators which show an intensity change only, namely, not a change in tint, or a negligible one, precision can be attained by the use of a Duboscq or Kober colorimeter. The depth of colour varies with the hydrogen ion concentration, but the pH scale is the negative logarithm of this. Over an interval of pH 0·2 it has been found

that the error from this does not exceed pH 0·01, namely, what is judged by intensity or tint to be pHx + 0·10 is in reality pHx + 0·09.

Ruppin has further calculated that a calcium carbonate solution in equilibrium with the carbon dioxide of the air, which exerts a partial pressure of 3×10^{-4} atmosphere, contains five times as much of the carbonate as does a solution in water free from carbonic acid at 16° C. For this the hydroxyl number is 17×10^{-7}, which, being converted, gives pH 8·37. This, then, is the reaction to be expected in a saturated solution freely exposed to the air. In the soil, however, the action of bacteria, protozoa, worms, fungi, and plant roots results in further production of carbon dioxide, so that the equilibrium is displaced in the more acid direction. Bjerrum and Gjaldbaek (1919) have determined by the electrical method that a solution saturated at 18° C. with both calcium bicarbonate and carbonic acid at atmospheric pressure is at pH 5·2. The more carbon dioxide and the more bicarbonate there is in the soil the more acid will be the reaction up to this limit. It is improbable that these conditions are ever realized in nature, nitrogen being present in preponderating amount in the soil atmosphere. The state actually occurring in the soil is not, however, true equilibrium, and carbon dioxide will be given off slowly into the soil air, which is always much richer in this gas than is the atmosphere. The importance of this has of late been emphasized by Howard and Howard (1920) and by Turpin (1920). The value 0·25 per cent. has been given by Keen (1921) as an average for the content of carbon dioxide in the soil air, but Howard has found up to 1·9 per cent. in badly aerated soils in the rainy months at Pusa.

Thus, considering the equilibrium between carbonate, bicarbonate, carbonic acid, and dissolved gaseous carbon dioxide and the disturbance caused by production of the gas in the soil, it is clear that the pH value of the soil solution is dynamic rather than static. This is very evident in soil extracts in which those rich in organic matters rapidly become less alkaline on standing owing to bacterial action. To attempt to measure the pH value of soils to the extremes of accuracy of which the method is capable is, therefore, in most cases waste of time from a biological standpoint, as it will vary with the temperature as affecting the life of organisms, with temperature as affecting the equilibrium quantity of carbon dioxide dissolved, and with the moisture-content as regulating the removal of carbon dioxide from the soil, as well as, indirectly, its production.

Wells (1915) gives the solubility of calcite in contact with the atmosphere as varying from 81 to 70, 61, 52, 44, 38 parts per million as the temperature rises from 0° to 50° C. in 10° steps. From these figures it

follows that as river-water rising in a cold region in contact with limestone flows to a warmer region it must deposit calcium carbonate and become more alkaline in the process, since as temperature rises the carbon dioxide content of the water is reduced, so that the value pH 8·37 at 16° C. is decreased at 0° C. and increased at 35° C. The hydrogen ion concentration of pure water varies, however, in the opposite sense. The water of the River Gandak at Pusa, in Bihar, India, was found to be at about pH 8·6, the air temperature being about 25° C. in November, falling from a much higher value in October. It may be remarked that sea-water is at about pH 8·2 in the Atlantic near the British Isles, but shows seasonal variations, due to photosynthetic action of plankton. The soil in many parts of Bihar, in the basin of the Ganges and its tributaries, is a fine silt, containing 10 to 40 per cent. of calcium carbonate. This soil, with remarkable uniformity, gives pH 9·0, or thereabouts, when freshly shaken up with water free from dissolved salts and carbonic acid. On standing, however, the pH value decreases owing to bacterial action. This deposition of calcium carbonate with the silt appears to stand in relation to the temperature changes undergone by the water in its progress from the cold mountain regions through the plains.

(b) *Magnesium salts.*—In the foregoing, calcium carbonate has been considered as the sole source of alkalinity. Many natural waters, however, contain magnesium carbonate. It has been shown by Moore, Prideaux, and Herdman (1915) and by M'Clendon (1917) that the major portion of the alkalinity of the sea is due to magnesium salts, on account of the greater solubility of magnesium carbonate, about 0·100 grams per litre, as against 0·0131 grams for calcium carbonate. Moore states that sea-water may become more alkaline than pH 9·1 owing to the removal of carbon dioxide by algae. The writer has found that it may even reach pH 9·7, viz., pH 9·95 with thymol phthalein uncorrected for salt error.

Corresponding with the greater solubility, magnesium carbonate, through hydrolysis, gives rise to a greater concentration of hydroxyl ions. Thus with cresol phthalein and thymol blue it was seen that a saturated solution was at least as alkaline as pH 9·6. Using as indicator thymol phthalein an exact match was obtained at pH 10·0, though this indicator is by no means at the end of its range at this point. From this it may be seen that neither soil extract nor natural water can surpass pH 10·0 owing to alkalinity derived from magnesium carbonate. Such a pH value would, however, be fatal to most, if not all, plants. Since calcium carbonate cannot give a value above pH 9·01, it is evident that higher alkalinity is possible where magnesium salts are present with calcium carbonate, as in sea-water.

The relation of these carbonates to marine plants will be discussed elsewhere, but it may be added that the photosynthetic activity of both marine and fresh-water algae or phanerogams is a very efficient process for removing dissolved carbon dioxide and bicarbonates (Angelstein (1911), Nathansohn (1907)), so that a point at or close to the maximum pH value for the carbonate in the solution may be attained provided this does not prove fatal to the assimilating plant. It may, however, be the means whereby one plant destroys another, as has been found to happen with *Ceramium rubrum* in presence of actively assimilating *Ulva lactuca*.

It is not, of course, suggested that a soil containing appreciable amounts of magnesium carbonate is at all as alkaline as pH 10, but this upper limit is higher than that of calcium carbonate. It is possible that the widespread dislike among agriculturists (see Hall, 1910; Russell, 1912; Aston, 1916) towards using limestone rich in magnesium carbonate may have some connexion with this. It has been shown by Hardy (1921) that dolomite retards nitrite and nitrate bacteria, which grow normally with calcium carbonate.

When other salts are present in addition to calcium carbonate the complex mixture may have appreciable effects upon the less soluble constituents. Thus when Merk's pure precipitated calcium sulphate was dissolved in cold water a solution of pH 7·9 was obtained; on boiling to decompose bicarbonate pH 8·3 was found. Thus the traces of carbonate present were insufficient to produce the maximum alkalinity pH 9·0. However, when equal volumes of calcium sulphate solution at pH 7·9 and calcium carbonate (bicarbonate) at pH 7·2 were mixed and boiled, the resulting reaction was over pH 8·8, which suggests a lessening of the solubility of the carbonate through the presence of calcium ions derived from the sulphate. This is conclusively proved to be the correct explanation by boiling water containing both solids in excess. The resulting solution when quickly cooled and tested was at pH 8·0 approximately.

Similarly magnesium sulphate, a good commercial sample, was found to give pH 8·0, rising to pH 8·5 on boiling. Kahlbaum's salt was, however, neutral in solution. On performing a qualitative test with the sulphate and carbonate after boiling pH 9·3 was not surpassed, and with the chloride pH 9·2, the differences doubtless being due to the amounts of sulphate and chloride having been added in small indefinite quantities. Magnesium nitrate was found to give pH 9·6; thus it must have contained an appreciable amount of carbonate. Again, magnesium phosphate in the cold gave pH 8·3, but on boiling this value decreased to pH 7·0, which, after an hour, only altered to pH 6·8; boiling for an extra half hour changed it to pH 6·5. A

sample of calcium phosphate gave in cold saturated solution pH 7·0, which on boiling for an hour and a half reached pH 5·7. From these examples it may be seen that, in making up nutritive solutions for plant-growth to which various nitrates, sulphates, and phosphates are added, a very indefinite reaction may result according to the purity of the salts used. Boiling to sterilize introduces further complications. M'Call and Haag (1920) have shown that nutrient solutions containing acid potassium phosphate have a lower hydrogen ion concentration than similar solutions with the acid phosphates of calcium or magnesium.

(c) *Action of gypsum on alkali lands.*—This type of study is, however, not merely of academic or laboratory importance, but has applications in the reclaiming af alkaline lands. In cases of extreme alkalinity sodium carbonate is present, often in considerable quantity. It is well known that gypsum is beneficial in counteracting the alkalinity. Now, even very minute amounts of sodium carbonate suffice to bring a solution to over pH 10, but by adding gypsum the carbonate is precipitated as calcium carbonate till the equilibrium is obtained for the system in contact with the carbon dioxide of the air. This results in the lowering of the pH value, as may be readily demonstrated. For this purpose a solution of sodium carbonate was made up, and was well over pH 10 as shown with thymol phthalein, which is the most useful indicator for this range, though unfortunately its blue colour is not stable. On adding a little solid calcium sulphate and warming for two to three minutes in a water bath the value fell to pH 8·4. A portion of the same mixture in the cold was at pH 9·4, but had fallen to 8·4 by the next day. Further standing exposed to the air resulted in both heated and unheated mixtures attaining to pH 7·8. Thus this very simple test enables one to study the progress of the action of gypsum or flushing, and to judge the quantities necessary in any given case. A soil which gives any blue with thymol phthalein is probably too alkaline for most crops, as this reagent gives no colour at pH 9·2, but a light blue at 9·4. Thymol blue (thymol sulphone phthalein) gives a blue colour down to about pH 9, and is yellow at pH 8. Cresol red may conveniently be used for the lower range of alkalinity. Soil is certainly fit for crops in India at pH 9·0, or possibly in a somewhat more alkaline state. It is of interest to note that in studying the relation of the calcium content of Kansas soils to the hydrogen ion concentration, Swanson, Latshaw, and Tague (1921) found values up to pH 8·6 for a loamy sand, and pH 8·40–9·03 for Oswego silt loam. These determinations were made by electrometric titration, and the value pH 9·03 is a high value for arable land. The soil originating from limestone in a quarry in Middle Devonian strata near Plymouth, was at pH 9·0 as a maximum. Wherry (1916)

records soil derived from limestone as giving pH 8·5, and that from dolomitic limestone pH 9·0, as determined colorimetrically.

(d) *Solubility of phosphates and occurrence of sulphur acids.*—Another point worthy of consideration is the effect of soil reaction upon the solubility of calcium phosphate. It appears probable that in the presence of calcium carbonate, bicarbonate, and sulphate the influence of the common calcium ion is such as to lessen the solubility of the phosphate. It is well known that the ordinary methods of estimation show low values for "available phosphate" in soils rich in calcium carbonate. Conversely, the action of an acid soil is to increase the available phosphate. Thus in Assam the soils of tea and indigo estates and jungle land have been shown by Davis (1918) to be rich in the latter. Samples, for which the writer is indebted to Mr. W. A. Davis, were found to be as acid as pH 5·4. In this case the acidity was clearly due to the oxidation of sulphur derived from iron pyrites, the latter being found in small grains in ten out of eleven samples; the pyrites had apparently all disintegrated in one surface sample. The possibility of the acidity being derived from organic sources is, it appears, excluded by the fact that the soil samples were not at all of a peaty nature, but of an open sandy texture, some sub-soils being just coarse sand. Furthermore, pyrites alone in water gives an acid reaction pH 4 to 5. Hall (1910) mentions cases where iron pyrites has been the cause of soil sterility, and states that such soils show an acid reaction. Alway (1920, 1), too, mentions a toxic layer of this nature under certain peat lands. Within the last few years much attention has been given to the action of sulphur bacteria in producing acid in the soil. The studies of Gillespie and Hurst and of Martin have already been mentioned in connexion with the control of potato scab. Further, Lipman, M'Lean, and Lint (1916) showed that the biological oxidation of sulphur in the soil produced soluble phosphate. Organic matter favours this, doubtless by providing additional acid and food for the bacteria. Brown and Warner (1917) demonstrated the production of soluble phosphate from rock phosphate by composting with sulphur and manure. Ames and Richmond (1918) and Shedd (1919) studied in addition the effect of nitrification. Tottingham and Hart (1921) found acidity as high as pH 2·9 and 3·1 with sulphur composts, and pH 3·4 and 3·7 with sulphur and rock phosphates. The general relations of inoculated sulphur as a plant-food solvent have been studied by Lipman, Blair, Martin, and Beckwith (1921). It is not, however, only by organisms that sulphur is oxidised. Thus MacIntire, Gray, and Shaw (1921) have shown that in sterile quartz media elemental sulphur is oxidised with production of acid. For its production from iron pyrites they give the following equation :—

$$FeS_2 + 3O_2 \rightleftarrows FeSO_4 + SO_2.$$

From the foregoing it will have been seen that the acidity found in soil is not necessarily due to humus or other organic acids. Of the actual occurrence of acid derived from sulphur, records are not, however, numerous. Beyond those cited by Hall and Alway, the writer has only met with (1) the suggestion by Wherry (1918) that the cause of the acid reaction pH 4·5 of certain spring waters in the United States is the presence of traces of sulphuric acid derived from iron pyrites; (2) the statements of Fischer (1914) and Kappen (1920), both cited from Odén (1921), in relation to the source of acid in humus; (3) the observations of acid water in Utah made by Lee (1907); and (4) the surprisingly great acidity, amounting to 1,360 parts per million of free sulphuric acid, found by Skeats (1902) in Texas. This acidity is slightly greater than N/40, which contains 1,225 parts per million, and corresponds to approximately pH 1·7. Clarke (1916) mentions no further observations; but it is highly probable that were spring waters in regions free from calcareous strata to be tested, the existence of natural acidity due to this acid would be found to be far from uncommon, even in districts remote from known sulphur deposits and the neighbourhood of volcanoes.

(e) *Solubility of iron salts.*—Soil reaction also regulates the solubility of iron salts. The corrosion of iron pipes by moorland water is well known, even though this water may be free from all acids other than atmospheric carbon dioxide, giving a reaction of pH 6·5 only. This appears to suffice to dissolve the metal with formation of ferrous bicarbonate, which is hydrolysed very largely, or almost completely. When, however, water is alkaline, there is much less solution of iron, and this is not without its effect on certain crops. The deficiency of available iron is sometimes considered as a cause of chlorosis, and it may be remarked that at Pusa, *Cajanus indicus*, a leguminous crop, which is much grown, often shows a yellowing of the leaves, the soil reaction being pH 8·7. The soil is a highly calcareous silt, but here the chlorosis may be due to causes other than the small quantity of available iron, for available phosphate is greatly reduced, as shown by Davis (1918). Gile and Carrero (1920) studied the cause of lime-induced chlorosis and the availability of iron in Porto Rico soils with regard to the rice crop. The ash of chlorotic plants was proved to be low in iron. They found that rice became chlorotic in calcareous soils with the ordinary percentage of water, yet would grow normally in certain calcareous soils when the soil was submerged. They state that calcifugous plants become chlorotic on calcareous soils, and advance as an explanation for the behaviour of rice in submerged soil the view that a new kind of root is developed which is better able to assimilate iron, the availability of which is decreased by the alkalinity To the writer, however, it appears that the change in the hydrogen ion

concentration of the soil when submerged may so increase the solubility of the iron salts or oxides present as to give the amount required. For, on imitating the conditions of submergence on a laboratory scale, it was found that the water standing over the soil in a test tube was at first as alkaline as pH 9·0. Even though shut off from external supplies of carbon dioxide, the pH value fell to 7·2 or 7·3 in less than six days, whilst in a day it was down to 8·1 in certain cases; in samples of soil from the top six inches the minimum values were reached more rapidly, as a rule, than in those from greater depths, namely, six to twelve, and twelve to thirty-six inches. In addition to this change in reaction, occasioned by bacteria, which are more numerous near the surface, it must be noted that owing to submergence and the organic matter present the soil is deprived of free oxygen, and so under the reducing conditions iron compounds will tend to pass into the ferrous state, in which they are more readily soluble. It is not, however, to be expected that the reaction of the soil solution itself will change with as great rapidity, or, perhaps, to as great an extent, for the calcium carbonate is continually being attacked by the free carbonic acid, and so a new equilibrium is reached. With excess of carbon dioxide there can, however, be no free carbonate in solution, while there must certainly be free carbonic acid, so the soil reaction must be, at least, no more alkaline than the bicarbonate stage. Bicarbonate gives only a trace of colour with phenol phthalein in boiled distilled water, and this indicator shows a trace of colour at pH 8·0 with Clark and Lubs standards, and a light pink at pH 8·2, the latter being the stage that would ordinarily be observed.

(f) *Effect of manuring upon soil reaction.*—Another point to be considered is the effect of manuring upon soil reaction, in particular with regard to what may be termed the unintentional alterations in reaction. Thus, while lime obviously tends to reduce acidity, and sulphur to increase it, it is not at all so obvious that ammonium sulphate and potassium sulphate should tend to increase it also. Yet the prolonged experiments at Rothamsted, and work elsewhere, have shown this to be so. How far these sulphates have affected the highly calcareous silt of the Pusa manurial series during the fifteen years it has been in existence is a matter for further research. It appears as if the reaction in the plots so treated is about pH 0·2–0·4 less than in other plots (Atkins, 1921); but as bacterial action in liberating carbon dioxide in the soil solution may be more rapid in the presence of these salts, it is uncertain to what extent the results are affected by this. At any rate, the effective pH value—that which prevails in the moist soil in contact with the plant roots—must be lower if bacterial action is more vigorous than in the untreated plots. Sodium nitrate, in which the acid radicle is taken

up, leaving sodium carbonate in the soil, tends to decrease acidity, but the writer has had no opportunity of measuring this effect.

In general, it is found that a garden soil supplied with stable manure in a limestone region in the British Isles gives a reaction of pH 7·0–7·6, though very rich in calcium carbonate, as shown by effervescence with acid. The same soil unmanured varies from pH 8·0 to as much as 9·0 in quarry subsoil. Dressing with seaweed probably tends to give an alkaline reaction on account of the surface contamination with sea salt and salty sand. Another method whereby the alkalinity may be increased or acidity reduced is by burning the surface growth. This well-known device leaves an ash of oxides and carbonates of the plant bases. In one instance examined, the burnt patch of grass in a field gave a good deep pink when tested with cresol phthalein, thus denoting a reaction of about pH·9, whereas the soil around the roots of the unburnt grass was at pH 6·5. The soluble ash alkalis are, of course, washed down by rain, and the bases again become available for plant growth.

(g) *Acid soils.*—So far, with the exception of an account of the existence of free sulphuric acid in soils, soil alkalinity has been discussed; that is to say, the soils with pH values of 7·0 or over. The exact neutral point varies somewhat with temperature, being 7·10 at 16°, 7·00 at 22°, and 6·71 at 40°. Odén (1921) has discussed soil acidity in special relation to the question of the existence of humic acid. He distinguishes between this problem and that of the cause of the acidity actually found, and has shown that preparations of well-washed humic acid give no greater acidity than pH 4·5, owing to the slight solubility of the acid. Since higher acidities may be met with in peat, it is obvious that other acids must be concerned in its production; in the case he gives the acidity of the peat was pH 4·09. Indeed, he quotes various workers to the effect that the extra acidity is due to such acids as sulphuric, phosphoric, acetic, malic, propionic, and others in very minute amounts. Just as calcium carbonate acts as a buffer in alkaline soils, and prevents the reaction being altered to acidity till it is all neutralized, so does humic acid act in acid soils. Odén illustrates this by giving a titration with sodium hydroxide of a peat suspension, noting the pH values after various additions. Unlike the calcium salts got from limestone, which are soluble and are washed out, the humates are relatively insoluble, and remain in the soil as regulators of its reaction.

Thus, to sum up, limestone soils are alkaline, the buffer action depending upon the percentage of carbonate and bicarbonate; peaty soils are acid, the buffer action being proportional to the amount of the humus acids of low solubility. There remain the sandy and clay soils, and these, if free from

carbonate, have less buffer action, especially the sandy. That is to say, percolation with an alkaline water renders them alkaline, with an acid water they become acid; normally they are feebly acid, due to carbonic acid and plant remains. Many are, however, not free from carbonates, and are as alkaline as are limestone soils, though having a lower buffer action. Furthermore, it appears that, owing to adsorption effects, clay soils may have a very considerable buffer action.

The hydrogen ion concentration of heated and unheated soil.

Numerous researches emanating from Rothamsted and elsewhere have shown how profound is the influence of heat in modifying the soil and its extracts, and in effecting changes, both in quantity and character, in the soil population. Recent bacteriology contains many records of the influence the hydrogen ion concentration exerts upon the growth of bacteria, protozoa, and fungi. It is accordingly important to consider how heat may alter the soil reaction.

Grigoriev (1916) noticed that the alkalinity of soil extracts increased during evaporation. Sharp and Hoagland (1919) pointed out that the effect of boiling was very slight or nil in the case of soil extracts lying between pH 5·3–7·3. This has been confirmed by the writer for sandy soil in the region of pH 5·4, which only altered to pH 5·7. But with alkaline soils giving extracts of pH 7·8–8·4 it was found that, on boiling, the carbonate limit, slightly over pH 9·0, was reached through elimination of carbon dioxide. With a soil from Bihar, originally at pH 9·0, when tested by shaking the air-dried silt with boiled distilled water, pH 9·2 was reached, due probably to the presence of traces of magnesium insufficient in quantity to give its maximum carbonate reaction, pH 10·0. Moore (1920) has pointed out that as a rise of pH 1 increases the hydroxyl ten times and diminishes the hydrion by the same factor, the result is a hundred-fold alteration in the relation between amphoteric colloids and their ions. This relation is of the utmost importance in interpreting the effect of changes in hydrogen ion concentration, since these are effective in proportion to their squares. The large alterations in reaction occasioned by heating the extracts of alkaline soils are probably not without a marked effect on the growth of bacteria, etc., subsequently in soil similarly treated. As multiplication progresses, however, the production of carbon dioxide restores the pH value to the neighbourhood of its initial value. How closely this is regained will depend upon how far the soil has been altered physically by heat treatment, which probably leaves it in a rather different state as regards aeration when brought back to its initial water-content. Further research on this subject seems desirable.

Nomenclature of soils with respect to acidity and alkalinity.

Wherry, in dealing with plant distribution in relation to the hydrogen ion concentration of the soil, felt the need of some verbal expression other than quoting pH limits. He accordingly (1919) proposed a convenient terminology which will be used in places in the account of such work which follows, but the pH limits by which they are defined should always be borne in mind. Thus from pH 7–6 is denoted as minimacid, 6–5 as subacid, 5–4 as mediacid, less than pH 4 as superacid. Corresponding terms are used in the alkaline range. This nomenclature is not entirely free from objection, especially on the alkaline side, as there is reason to believe that pH 9·0 up to 9·2, or possibly more, is not as a general rule harmful to plants and certain protozoa, while it is exremely likely that pH 9·7 and over is decidedly injurious to all plant life; yet both are classed as medialkaline, and only at pH 10 is superalkaline reached. Wherry also drew attention to the fact that pH values were not understood by many biologists, and suggested the use of such terms as specific acidity and specific alkalinity, taking the hydrogen and hydroxyl ion concentration of pure water as one. Thus pH 6 is of specific acidity 10, pH 5·5 approximately 30—in reality it is 32—and is given by Wherry as 30 +. Correspondingly on the alkaline side, pH 9 is equivalent to specific alkalinity 100. To the writer these specific values appear to be unnecessary, and to destroy the unity of the one scale, the pH value, which covers all ranges of chemical and biological reaction. Thus, if figures are given, they will be pH values, as is strongly advocated by Clark (1920).

In addition to the terms already given, Wherry embraces the region from pH 6–8 in the term circumneutral. In this limit he places leaf mould, whilst bog peat is from pH 6 to 4 approximately, and "alkali" soil from pH 8 upwards. Upland peat is given as pH 5–7, and limestone soil as 7–9. Fischer (1914), by the electrical method, found for "Hochmoor" sphagnum pH 3·02–3·19, and for "Flachmoor" soil and plant débris pH 5·22–5·95.

A proposal similar to Wherry's was made by Walker and Kay (1912) some years previously for recording the alkalinity (or acidity) of water in terms of specific values, taking pure water at the same temperature as unity. This was made before the use of the pH symbol became general.

Relation of the hydrogen ion concentration of soil to geological formation originating it.

It is obvious that when a soil has been transported so as to lie over strata of different composition from those from which it originated, there can be no relation between the acidity or alkalinity of the soil and rock strata.

From what has been said already it is evident that the supply of bases capable of neutralizing plant remains is the primary factor which regulates soil reaction. In an interesting series of papers, a list of which may be found in the bibliography, from 1916 onwards Wherry studied the relations of the strata to the soil and of the latter to plant distribution. Thus granitic and quartzite rocks have no bases free to act in neutralizing acidity. Slaty rocks are in much the same position, and so are many sandstones. Probably some slates contain a minute amount of carbonate, and sandstones, especially when porous and underlying limestone, may contain notable amounts. The reaction that deeper strata are capable of giving may be indicated by springs, as shown further on.

The following observations may serve to illustrate the relationship. The determinations were made by the colorimetric method, using about a gram of soil to 10 c.c. of water, and testing the approximately clear supernatant liquid. If turbid, the device was adopted of viewing the standards through tubes containing the turbid extract without indicator, the extract of the sample containing indicator being viewed through water to compensate. As explained by Clark, dilution in many cases has not a large effect upon pH, and this was tested with a fine calcareous silt by using only one-fifth the usual quantity of soil and obtaining an indistinguishable colour. The advantage of thus obtaining a clearer solution was obvious. A slight turbidity often increases the buffer action of the solution and lessens errors. With acid soils it appears that larger quantities of solid are necessary to avoid dilution effects. It has sometimes been found convenient to mix the soil with a slight excess of water in a watch-glass, and to test a drop draining away against a white background. As explained by the writer in describing the determination of the acidity of plant tissues, use is made of drops of the standard solutions, or even of the latter rendered turbid by the soil extract. Since the extract has relatively slight buffer action, and that of the standard is intentionally very considerable, the latter is not seriously altered by this; and if identical in pH value with the sample, the error is obviously zero. As a rule, two, or even three, indicators were used in fixing the pH of a sample.

In addition to the indicators given in their final list by Clark and Lubs, it has been found that di-ethyl red—also tested by these workers—is very useful for a region just below that of methyl red. Di-ethyl red changes from yellow to red between pH 6·0 and 5·8, and is increasing in colour just where methyl red is yellow, and where brom cresol purple is turning yellow also. A red colour is very easily observed in a turbid solution, so the value of di-ethyl red in checking the results given by other indicators is

considerable. For the extreme alkaline range, pH 9·4 to 10·0 thymol phthalein is quite the best indicator, though it fades quickly.

In the district round Plymouth, in the counties of Devon and Cornwall, the oldest Devonian formations are on the coast, and the newer are encountered as one proceeds inland in a northerly direction. There are, however, numerous isolated patches of rocks of igneous or plutonic origin.

Thus in Cornwall, near Rame Head, to the west of Plymouth Sound, are found the Dartmouth Slates, in the Lower Devonian. They also extend across to the eastern side. The soil is a dark chocolate brown, and gave the reactions pH 6·7–7·3, as shown in Table I. This siliceous rock yields a soil which may be caused to become acid or alkaline; it appears to have but little buffer action. Some of the results for fields may be due to the addition of limestone as a manure; those parts near the coast—and none are far from it—may be enriched and neutralized by the shells of mussels, periwinkles, and such like brought up by birds, by the use of sea-weed as manure, and by the strong southerly winds carrying minute amounts of sea-spray in suspension. These factors all appear to lessen the production of strongly acid soils near the sea, and they are by no means negligible in their action over prolonged periods. Inland from the Dartmouth Slates come the Staddon Grits, which consist of reddish grits and some slates and shales, with thin limestone bands.

This formation runs east and west on both sides of Plymouth Sound, as shown in the maps of the Geological Survey. The rocks and soil derived therefrom are of a deep red colour, and stand in marked contrast to the Dartmouth Slates, the soil from which is dark brown. In the table may be seen the results given by this formation. It appears to be of definite, but low, alkalinity, except under special conditions, such as where pine needles make up most of the superficial soil, which may be as acid as pH 5·2, and on slate. Apparently these coarse sandstones have become permeated with calcium carbonate from the limestones which originally rested over them. Whatever the cause, the soil is but little less alkaline than that lying directly on limestone, provided it is not practically disintegrated limestone.

The line of separation of the Dartmouth Slates and Staddon Grits lies in the bay containing the Cawsand and Kingsand village. Just to the north of this, on the sound, and for about a quarter of a mile inland, lies a patch of felsite. On this the soil is blackish, and everywhere acid, save in a small glen, bounded on two sides by Staddon Grit, where drainage water and downward movement of soil have resulted in a sufficient admixture of calcium salts to neutralize the acidity. This appears a fertile spot, and is divided up into allotments.

Immediately to the north of the Staddon Grits lie the Mid-Devonian Slates, followed by the Mid-Devonian Limestone. The soil yielded by the limestone varies from near neutrality to an alkalinity of pH 9·0 in quarries and rocky subsoil. Thus it is possible to find soil of about pH 7·6 in both Staddon Grit and the limestone formation. In the Mid-Devonian Slates the soil near the northern limit, viz., next the limestone, is alkaline, pH 8·4, whereas the lower beds give acid soil, pH 6·0–6·8.

Here and there in most of this region there are small patches of Devonian volcanic rocks, which in places are schalstein. This calcareous rock appears to give an alkaline soil also. To the north of the limestone lies a great expanse of Upper Devonian Slate, dotted with volcanic rocks. These slaty rocks yield a soil which, like the Dartmouth Slates may be on either side of neutrality, according to situation and exposure. Thus, whilst there is never enough calcium carbonate to bring the alkalinity up to that of limestone soils, on an average, there appears to be enough, in parts, to prevent the development of any considerable acidity. The Compton district is marked in the map as being schalstein and tuff, hence its greater alkalinity than that of Egg Buckland, where the Upper Devonian Slates are developed. This formation extends northwards for a number of miles, until the metamorphic rocks adjacent to the granite appear. A stream flowing from Compton, below Egg Buckland, is at about pH 8·0, pointing to the existence of calcareous rock, whereas the Dartmoor water is at about pH 6·8.

Northwards again lies Dartmoor, a granite rock covered by peaty soil and siliceous débris. Here, as far as the writer's examination has proceeded, the soil is acid, mostly pH 5·4 to 5·1. It is particularly interesting to note how this acidity extends deep into the subsoil among the débris of the rock. The most acid soil found was that in the crevices of a loosely piled granite wall, which must have been formed almost exclusively of plant remains. This soil at pH 4·1 falls in Wherry's nomenclature into the mediacid group, the general reaction being subacid.

Thus, as one passes inland from the coast to Dartmoor, a very varied succession of soil reactions is encountered. It must not be thought, however, that a district is of uniform reaction; one can only say that it is predominantly of one reaction, as will be seen when considering the relation of the hydrogen ion concentration to plant distribution. For example, Wherry (1920) found that *Sarracenia spp.* abounded in the Dover swamps of Warren County, where the reaction was acid, but in a neighbouring swamp, Green Pond, the water was alkaline, and *Sarracenia*, though common, was only found on hummocks of plant remains, mosses, &c., in which the reaction was acid.

In contradistinction to the acidity developed on granite and felsite stands the alkalinity found in sand dunes and shingle near the sea. Here the

combination of sea-salt, comminuted fragments of shells, and the porous nature of the sand, which permits the removal of bicarbonates, results in a reaction which is always alkaline, often markedly so up to near pH 9·0. To this probably the high temperature reached by sand in the sunlight also contributes, as the bicarbonate may be partly decomposed by this.

It may be added that the Devonian Slate cliffs at Pentire are, in spite of their neighbourhood to the sea, slightly acid, pH 6·6–7·0, whilst the pillow lava or spilite forming the extremity of Pentire Head yields a slightly acid soil, pH 6·8. It is probably due to the wind-borne spray that the acidity is not greater.

It seems possible that within limits the determination of hydrogen ion concentration given by the soil or by the rocks themselves may be of some use in working out relationships of displaced strata where fossil evidence is wanting or scanty. Thus, a slaty or sandy soil may be acid in certain situations, but it is its limit on the alkaline side that may serve to distinguish it.

In this work use has been made of Ussher's (1907) memoir on the Plymouth district.

TABLE I.

Geological formation.	Nature of rock.	Locality.	Soil.	Typical pH values.
Lower Devonian.				
Dartmouth Slates, .	Slaty, . .	Rame Hd. and inland.	Light soil, dark brownish.	6·7–7·3
Staddon Grits, .	Sandstone, . .	Staddon Heights and Maker.	Sandy, deep red,	7·1–7·8
,, ,, .	Slaty, . .	Staddon Heights,	Light soil, brownish.	5·4
Intrusive, .	Felsite, . .	Kingsand, .	Shallow blackish soil, with plant remains.	5·4–6·4
Middle Devonian, .	Slate, . .	Staddon, . .	Light, brownish yellow soil.	6·0–8·4
,, ,, .	Limestone, . .	Plymouth district,	Somewhat sandy, light brown to black.	7·4–9·0
Upper Devonian.				
Volcanic and slates,	Schalstein and slates.	Compton, .	Brownish soil, light texture.	7·4
Upper Dev. slates, .	Slaty, . .	Egg Buckland, .	Dark brown light soil.	5·8–7·0
,, ,, ,, .	,, . .	Pentire Hd., .	,,	6·6–7·0
Volcanic, .	Pillow lava (spilite),	Pentire Hd., .	Shallow light soil,	6·8
Plutonic, .	Granite, . .	Dartmoor, .	Disintegrating granite.	5·2
,, .	,, . .	Glencullen, Co. Dublin.	Purely peat, .	4·6

The relation of the hydrogen ion concentration of the soil to plant distribution.

Wherry (1916) investigated the distribution of "the walking fern," *Camptosorus rhizophyllus*, in relation to the content of calcium salts in the soil. This was reputed to be a calcicolous species. He was unable to find any correlation, and sought for an explanation in terms of the hydrogen ion concentration. The habitats examined, however, gave a very extended range of values, from pH 5·5–9·0. In his further papers, which are of great interest, he traced the limits for numerous species of native orchids, for the Ericaceae and associated plants, and showed how it comes about that plants typical of the New Jersey Pine Barrens are found bordering a salt marsh. The distribution of species of ferns was also studied by Wherry, who pointed out the bearing that these researches have upon horticultural problems. He thinks it possible to work to an accuracy of pH 0·2, or in the field to pH 0·5. This, however, is considered entirely adequate, for from one plant to another, or even from one root to another, on the same individual observations may differ by pH 1. The writer would, however, point out that Wherry probably means this to hold only over certain ranges of reaction, for in his own experience in acid soils of a particular region great uniformity, to pH 0·2 or less, may be met with. The same is true of the more alkaline soils. But with slightly buffered soils near neutrality varying amounts of carbon dioxide have a relatively large effect.

Some plants appear to have narrow limits of growth, such as certain orchids and heath plants, with which special fungi are associated, as mycorhiza. Again, leguminous plants with root nodules are in their distribution possibly limited by the pH values the bacteria can endure in the soil.

The species studied by Wherry in the U.S.A. are for the most part absent from the British Isles, and the difference in climate may also to some small extent alter the reactions of various situations, so it is of interest to study the problem afresh.

An elaborate contribution to physiological ecology has also been made by Arrhenius (1920), who records not only pH values for different plant associations, but also moisture content, organic matter, calcium, potassium, total nitrogen, nitrate and ammonium nitrogen, and phosphate. The original should be consulted for its valuable details.

In the account which follows, the pH values given for a plant are either from separate districts, or from a quite different soil or situation in the same district. In the field one cannot fail to be impressed with the absence of certain plants from what would appear to be suitable situations; an explanation is often found when the reaction of the soil is examined. As a general

rule the records relate to common and widely spread plants, for it is reasonable to suppose that the absence of these is in any region due to some very definite factor, whereas with the rarer plants it is rather the concurrence of a number of favouring circumstances that renders growth possible. A few of the latter have, however, been mentioned.

With regard to the limitation of the distribution of the weeds of arable land to particular types of soil, a perusal of the lists given by Brenchley (1911, '12, '13) leaves one with the impression that it is hard to draw definite conclusions on the subject, the same plant being met with in very varying soils. Thus, a typical plant of limestone soil may be found in sandy soil, and on examination it has happened that the two soils had closely similar pH values, so far as the writer's experience has gone as yet.

When considering the relation of plant distribution to the hydrogen ion concentration of the soil solution, it is well to avoid the chastisement with scorpions of the modern ecologist by recalling a paper by Foot (1865): "All old floras dogmatically tell us Digitalis avoids the limestone. It is, doubtless, more plentiful on sandstone and clay formation, but grows on limestone also. It is abundant about Mullingar, also occasionally in Burren." The limits given for a species are accordingly only to be taken as embodying the results obtained so far. They do not mean that the species is never found in nature outside these limits; still less do they mean that it cannot grow in water culture outside the limits given. Growth in soil is complicated by many factors. Thus, Hartwell and Pember (1918) have shown that acid soil affects barley and rye differently, and that this is due to the aluminium salts, which do not harm the rye, but injure the barley. The addition of acid phosphate made the soil more acid, but the barley then grew well, as aluminium phosphate was precipitated. Miyake (1916), too, showed how toxic minute traces of aluminium salts were to rice. The effect of the acid is clearly an indirect one in these as in many other cases. This has recently been emphasized by Mirasol (1921), and Comber (1921) instances the different behaviour of a clay soil from that of a peaty soil of equal hydrogen ion concentration. But bearing in mind such very necessary limitations to its application, it is still true that the hydrogen ion concentration may be taken as an index of a set of conditions, physical and chemical, which control plant distribution. Thus, in a soil rich in calcium, the pH value is an index of soil aeration. When this is good the soil will give a reaction close to that for calcium bicarbonate in equilibrium with the air, pH 8·3–8·4, but as aeration becomes less and carbon dioxide accumulates in the soil atmosphere to the extent of 0·25 to one per cent. or over, the pH value decreases. Where there is only a small amount of bicarbonate the soil may even become acid from this cause. The

effects of "sourness" in soil must not therefore be thought of as due merely to the acidity, which may be trifling, and far less than that of the cell-sap of the roots. They are occasioned rather by a series of related causes, such as accumulation of carbonic acid, possibly other acids, and of reducing agents (Gillespie, 1920), deficiency of oxygen, and, in special cases, deficiency in calcium salts and inhibition of the beneficial action of various soil bacteria. What these conditions are for calcareous soils has recently been the subject of a lengthy survey by Salisbury (1920).

From what has been said already, it may be deduced that variations in soil reaction are more likely to be found in sandstone and slate districts than in those where limestone predominates. The buffer action of the carbonate-bicarbonate system prevents wide divergences. The reaction may vary from about pH 8·8–9·0 in a limestone quarry or near the rock to about pH 7·5 in a field, or even less in a well-manured garden. A very average value is in the neighbourhood of pH 8. In sea sand the comminuted shells give a continuous supply of calcium salts long after much of the soluble sodium salts has been washed out. Fisher (1921, 2) has drawn attention to the importance of the buffer action of the soil, and instances the action of basic slag in altering the reaction of a light sand, and not of a heavy loam.

The soils of the province of Bihar, India, illustrate the uniformity of reaction due to calcareous silt. Samples from various portions of the estate of the Agricultural Research Institute, Pusa, were at pH 8·6–9·0, and identical values were given by soils from indigo estates in this province, from different fields in each estate, and at depths of 0–6 in., 6–12 in., and 1–3 ft. Since, in addition to a number of Pusa samples, thirty-six others from three estates were examined, the uniformity of the reaction is evident. Obviously a study of plant distribution in such a district does not extend beyond one type, the calcicolous. A good variety, however, may be obtained in the Youghal district, Co. Cork, and the adjacent portions of Co. Waterford.

A road runs approximately north and south from the seashore through some low-lying sandy country, which is also boggy in parts. To the east on the sea-front is "Clay Castle," a sandy cliff with patches of hard clay; to the west are dunes bordering the rifle range. Further inland are fields under grass, potatoes, turnips, &c., especially along a cross-road to the east. Beyond the latter a glen is entered, with red sandstone quarries; in this the road rises considerably to about 150 or 200 feet above sea-level. Within this limited area, bordering a distance of something over half a mile on the road, the soil reaction varies from pH 8·8 to 5·6, and diverse types of vegetation are found.

Nearest of all plants to the sea one comes across *Salsola Kali.* The sand round its roots gives a solution which is slaty blue with thymol blue, denoting the value pH 9·0. This must be corrected for neutral salt error ; the correction is approximate, and reduces the value to pH 8·8. This extract also gives a good pink purple with cresol phthalein, an indicator which shows no trace of colour below pH 8·2. The reaction is more intense than the sand encrusted by the sea, which was at pH 8·4. On the clayey sand of the face of Clay Castle, *Lotus corniculatus* and *Anthyllis vulneraria* flowered in great profusion, as did also *Erodium cicutarium*, the reaction being pH 8·3.

The sand dune to the west was at about pH 8·4 on the outer side, where were found *Salsola Kali* and *Arenaria peploides*, followed by other plants characteristic of mobile dunes—*Elymus arenarius, Euphorbia Paralias, Eryngium maritimum.* Slightly further in were found *Sedum acre, Convolvulus Soldanella*, and then big patches of *Trifolium repens* and *Lotus corniculatus* at about pH 8·0–7·8, with some *T. pratense* also. To the east the higher ground was grassy, and the sandy soil was evidently capable of but slight buffer action, bare portions among shingle with *Arenaria peploides* giving pH 8·0, and the grassy portions pH 7·2–7·8 with *L. corniculatus, Plantago Coronopus, Taraxacum officinale, Salvia Verbenaca, Ononis arvensis, Erodium cicutarium* L'Hér. var. *hirtum*, etc. Somewhat further inland the higher ground was at pH 8·2, with on the banks *Linaria Cymbalaria, Achillea Millefolium, Galium verum, Bellis perennis*, and further on occasional bushes of *Crataegus Oxyacantha* and *Ulex europaeus.*

Immediately behind Clay Castle the land slopes northwards to a marshy depression. On the slope the soil has more clay mingled with the sand, and no longer gives any effervescence with hydrochloric acid. Its reaction is pH 6·4, as shown by brom thymol blue and brom cresol purple. Here in the dryer portions *Ulex europaeus* is plentiful, and in the wet parts *Iris Pseudacorus, Lychnis flos-cuculi, Orchis sp., Eriophorum sp.* The low portions, where the bog-cotton occurs, merge into a marsh. Here the reaction was found to be pH 7·2, and *Cardamine pratensis* was abundant. It may be mentioned that among the gorse on the higher level were several dumps of rubble and a sandy earth at pH 7·6, the ground around being at pH 6·4. On the dumps were *Bellis perennis, Anagallis arvensis, Sinapis sp.*, and *Malva sylvestris*, none of which were noticed on the acid soil.

The marshy region adjoins the road. On proceeding northwards an embankment facing west was largely covered by *Rubus fruticosus*, abundant grass with a large white clover, *Convolvulus arvensis*, and *Equisetum arvense.* The reaction hereabouts is close to pH 8. The road shortly enters the glen,

but, before following it there, a digression may be made eastwards to study the banks and arable land bordering the cross-road.

Here on the walls facing south the most inhospitable-looking crevices in the mortar are occupied by *Parietaria officinalis*, and give a reaction of about pH 8–8·2. The earth-covered tops of the wall give much the same reaction, and are covered by *Veronica arvensis, Euphorbia Peplus, Taraxacum officinale, Bellis perennis, Senecio vulgaris*, with an odd clump of *Malva sylvestris*, and further on *Capsella Bursa-pastoris* at pH 7·8 or thereabouts. Immediately inside the wall in the grassy ditch were *Convolvulus sepium, Lychnis vespertina*, and *Symphytum officinale*. The reaction both here and in the field was about pH 7·6. The field contained potatoes in part, and was full of weeds, such as *Fumaria officinalis, Euphorbia Helioscopia, E. Peplus, Polygonum aviculare, Papaver Rhoeas, Veronica arvensis, V. agrestis, Scabiosa arvensis, Brassica campestris.*

On proceeding up the glen a by-road to the east is flanked by *R. fruticosus*, with *C. sepium*, *Urtica dioica*, and some *U. europaeus*, and among the grass *V. arvensis*, the reaction here being close to neutrality, pH 7·2.

Round the stream in the glen the ground is in places marshy, with rushes, *Nasturtium officinale, Polygonum Persicaria, Taraxacum officinale*, the reaction being pH 7·0. The water of the stream, however, was at pH 8·3, close to the value for bicarbonate in equilibrium with the carbon dioxide of the air. Two shallow wells beside it were at pH 6·4 and 6·6, due to excess of carbonic acid, as was shown by insolating the water with filamentous algae growing in it. Photosynthesis then rapidly removed the acid, the alkalinity rising above pH 8 in a few minutes.

The hillside on the east was characterized by the abundance of *Pteris aquilina*, with *Ulex europaeus* and *Rubus fruticosus*. Here the soil was markedly acid, pH 5·6 or 5·4.

On the other side of the road was a ruined cottage and several old red sandstone quarries. Round the cottage the soil was alkaline, about pH 7·8, and supported *R. fruticosus, Convolvulus sepium, Urtica dioica, Symphytum officinale, Anagallis arvensis, Polygonum aviculare, Viola tricolor*. In the quarries the scanty soil in the clefts and ledges was from pH 5·6–6·0. In testing these acid soils without standard solutions use was made of methyl red, di-ethyl red, brom cresol purple, and brom thymol blue. Memory of the tints these indicators give with standards, aided by Clark's coloured chart, enables a considerable degree of precision to be obtained. The clefts and ledges were occupied by *U. europaeus, Cotyledon Umbilicus* (which also grows in among mortar at pH 8), *Silene maritima* With., *Sedum anglicum*, with one clump of *Erica cinerea*. The lower parts with more soil had *Prunus*

spinosa, Veronica arvensis, Fumaria officinalis, and *Achillea Millefolium.* The Veronica and following two plants are usually found on alkaline soil, whereas here it was acid, pH 6·0.

Further examples of lowland acid soil are furnished by the banks of the Blackwater. On the western side the banks slope down steeply to the road. They are wooded, the trees being conifers, oaks, and mountain ash for the most part. Here the soil is peaty and as acid as pH 4·6, grading down to pH 6·4 in the ditch beside the road, a distance of a few feet. The peaty soil is covered with *Erica cinerea* and *Calluna vulgaris.* A certain amount of earth was, however, mixed with it in most parts the reaction being about pH 5·0 with both varieties of heather, *Scilla nutans, Pteris aquilina, Vaccinium Myrtillus,* and on rocky ledges *Polytrichum commune* and *Peltigera canina,* also at pH 5. A drop of four to ten feet brings one to the grassy bank bordering the road. Here are found *R. fruticosus, P. aquilina, Jasione montana, Viola canina, Lonicera Periclymenum,* with stray small plants of *Erica cinerea* and *Calluna vulgaris*; this soil, which is more earthy than peaty, is at pH 5·8. Further along the same bank was at pH 6·4, and supported *U. europaeus, J. montana, Aspidium sp.,* also *Agrimonia Eupatoria,* var. *odorata,* Mill. The absence of heather and bracken may be noted. The drain by the road was also at pH 6·4, and the bare earth was dotted with *Bellis perennis* and a small white clover. On the opposite side of the river the flora was very similar, though the sloping fields were not wooded at the spot examined. The earthy soil was at pH 5·8 on the bank and pH 5·4 in a dry field, from which both *Calluna vulgaris* and *Scilla nutans* were absent.

Upland acid soils may be illustrated by the peat of Glencullen at about 1,200 ft. altitude in Co. Dublin. This peat is at pH 4·6; both *Calluna vulgaris* and *Vaccinium Myrtillus* abound. One sample from Crib Goch, Snowdon, was at pH 5·0, the soil being earthy rather than peaty, and covered with grass, lichens, and also *Lycopodium Selago.* For these two soils the writer is indebted to Mr. L. B. Smyth.

Samples from Dartmoor were found to be very uniformly at pH 5·4–5·1, and were carefully compared against drops of standard buffer solutions on porcelain. Thus, at Staple Tors, both the drier earthy soil supporting *Erica Tetralix* and *Anagallis tenella,* and wetter portions with *Sphagnum sp., Narthecium ossifragum,* and *Drosera rotundifolia,* were at pH 5·4. At Merrivale, about a mile away, an earthy soil with plant remains was grass-covered in the drier parts, and as acid as pH 5·2, the wetter parts with *Eriophorum polystachion* and *Sphagnum sp.,* being at pH 5·4. The most acid soil as yet encountered was obtained from the crevices in a wall of piled granite here. On it grew mosses, a liverwort, and *Jasione montana.* It

turned methyl orange-red, and gave a slaty blue with brom phenol blue, corresponding to pH 4·1. Further on, near Two Bridges, an excavation exposed a granite subsoil. At a depth of three feet this was pinkish in colour, and at pH 5·4, whereas the blackish soil above it at a depth of 4–6 in. was at pH 5·2. In this region were upland pastures, and *Digitalis purpurea* was plentiful. More than a mile up this valley lies Wistman's Wood, a small grove of stunted oaks, gnarled to an extraordinary degree, and covered with lichens. The trees grow between large granite boulders, among which *Vaccinium Myrtillus* and *Sedum anglicum* abound. The soil reaction here was pH 5·1. In the approaches to the wood *Pteris aquilina* is dominant, and the pH value is, doubtless, closely similar, but was not tested.

The flora of sea cliffs was studied mainly at Polzeath and Pentire Head, on the north coast of Cornwall. The coast-line soil is on slaty rock, except at the extremity of the head, where the rock is "pillow lava." In reaction it varies from pH 6·6 to 7·0; the agencies which prevent the development of greater acidity have already been mentioned. The elevation reaches 300 ft. as a maximum, and in the more rocky portions are found *Silene maritima, Armeria vulgaris, Spergularia rupestris, S. rubra, Sedum anglicum.* The sloping banks, on which the grass had been withered by the drought, when seen at a small angle were blue with *Scilla autumnalis*; bushes of *Ulex europaeus* were found on the banks also, together with *Galium verum*, and very small plants of *Plantago Coronopus.* Nearest the sea, and apparently splashed by it at times, were found *Crithmum maritimum, A. vulgaris*, and *S. rupestris*, the reaction of the soil in the crevice being pH 8·2. At Youghal the two latter plants were similarly located at pH 7·2–7·6, but spray would at once raise the alkalinity.

In gullies, where streams ran in, the blackish-looking soil was found to be mixed with sea sand, and the boggy parts were at about pH 7·6, prominent plants being *Convolvulus sepium, Mentha aquatica*, and *Epilobium parviflorum.*

Along the coast at Pentire and Polzeath *Sedum anglicum* abounds, but *S. acre* could not be found. As previously mentioned, this region is at pH 6.6–6·8 mostly. Inland, however, about half a mile away, *S. acre* was found on a wall, the reaction being pH 7·8. Since *S. acre* occurs on sand dunes also, and *S. anglicum* on Dartmoor, neither proximity to the sea nor elevation can be considered as limiting factors. One is forced to the conclusion that the hydrogen ion concentration is in this case undoubtedly the factor which determines which of these two species occupies a situation.

Another rather striking instance of such a limitation is shown by *Centranthus ruber.* This has not been observed at less than pH 7·4 on a cliff, but on embankments round limestone quarries it is often the dominant,

if not the only, plant, the reaction being close to pH 9. Near Plymouth an embankment carries a road up to and past Stamford Fort. The south side of this embankment was thickly covered with *C. ruber*. The plant, however, ceased to appear beyond a certain vertical line, save for half a dozen stragglers within ten or twelve feet. Beyond this *Achillea Millefolium* and *Linaria vulgaris* were plentiful, also *Taraxacum officinale*, which occurred in the *Centranthus* area too. On examining the soil, it was found that the latter was at pH 9·0, and on standing with water for a day it only fell to pH 8·8. It had rather more clay-like particles than had the *Achillea* region, the reaction of which was no more than pH 8·2. On standing for a day with water this fell to pH 7·6, showing that the amount of carbon dioxide produced by bacteria was not taken up by excess of carbonate, as in the other sample. Evidently one portion of the embankment was made with limestone rubble from Turnchapel quarries, and the other from a slate near its end, which develops a reaction of pH 8·2 or over, and underlies the Middle Devonian Limestone.

Another point of interest, which, however, requires further work, is the difference in distribution of three species of Convolvulus, all very similar in general habit. *C. Soldanella* occurs on dunes and sand banks where water is not plentiful; aeration is very good, and the pH value 8·0–8·4 denotes the bicarbonate region with but little carbon dioxide. *C. arvensis* occurs in dry situations in fields and on banks, the recorded values being pH 7·6–8·2; these are reinforced by experience of other situations not examined. This range denotes bicarbonate with less efficient aeration and more carbon dioxide. Finally, *C. Sepium* occurs in hedges, in damp thickets, and as a garden weed, the range observed being pH 7·2–7·8. This corresponds with, on the average, rather more carbon dioxide than for *C. arvensis*, namely, with a damper situation.

Certain heath plants are obviously limited in their distribution also; thus *Calluna vulgaris* has been found abundant, at pH 4·6–5·2, on peat or peaty soil. *Erica cinerea*, however, is found in abundance at pH 4·6–5·4; also in drier situations, at pH 5·4, in earthy soil. *Vaccinium Myrtillus* has the distribution of Calluna rather than of Erica. The limits for sparse occurrence are somewhat wider.

Again, *Pteris aquilina* is usually found on acid soil; thus, out of ten situations, six were at pH 5–6, three at pH 6–7, and one at pH 7·6. The latter was certainly a spot rich in Pteris, but it was in a gully below land, at pH 6·4, where the plant was abundant.

In this description, and in the table which follows, dominant means plentiful to the more or less total exclusion of other plants; abundant means

that numerous patches or groups of the plant occur; and plentiful that numerous isolated plants are found.

Ulex europaeus is widely distributed, but its occurrence is mainly in the acid region. Thus, out of twenty-seven situations, five were from pH 5·4 to under pH 6, sixteen were from pH 6–7, two pH 7–8, and four pH 8–8·6. Of those beyond pH 7, only one at pH 7·2 was an abundant situation, and two were single bushes only. The remaining three were situations with a number of isolated bushes. It is plentiful on soil above limestone around Cork Harbour, but no opportunity of examining these regions has arisen. It is possible that the soil is boulder clay not derived from the limestone.

Rubus fruticosus must be placed among plants with a wide range, having been found between pH 5·4 and 8·4, being distributed abundantly through the whole of it. However, Briggs (1880) records thirty-eight sub-species in his Flora of Plymouth, and twenty-two are recognized by Hooker (3rd ed., 1884), not including *R. saxatilis*, which is given as a species. It is probable that a study of the varieties would show a less extended range.

The foregoing outline may be amplified by reference to the alphabetical list of plants in Table II, which records the pH limits. It is hoped that both species and situations may in the future be examined in greater number.

So far no reference has been made to the reaction of the soil carrying crops, since their occurrence is directed, and not natural. It is, however, a subject of much importance. One can instance the work already mentioned on the occurrence of potato scab; a paper accompanying this points out a relation with the "finger and toe" disease of turnips and other Cruciferae. It will be of interest to see whether many of this group are unable to thrive in soil even slightly acid; the majority are recorded by Briggs as occurring in localities known to the writer to have predominantly alkaline reactions, many exclusively so. If an alkaline region is acid in any part, this is either a boggy spot or a steep bank from which bicarbonate is readily leached out. Since it is laborious and costly to alter the reaction of the soil to suit the crop, the alternative is to choose the crop to suit the soil reaction, and this is being done to a considerable extent in Sweden, Germany, Austria, and the United States, where, in Minnesota, for example, as described by Alway (1920, 1 & 2), peaty soils are being brought into cultivation by supplying their deficiency in phosphates and sometimes in potassium, the crops most commonly grown being grasses for hay, onions, celery, lettuce, potatoes, beets, etc. The subject is undoubtedly worthy of greater attention in Ireland, as such large areas of peaty land occur. As mentioned previously, an acid peaty soil differs in important respects from a clay soil of equal hydrogen ion content.

It may be added that in Bihar *Indigofera arrecta* grows before the first cutting to about four feet. The reaction is about pH 8·7 or over, and available phosphates are low in many places in the calcareous silt. In parts of Assam, when grown on certain tea gardens with an open sandy soil and an abundance of available phosphate, the indigo grows luxuriantly, reaching ten feet before the first cutting, and twelve to fifteen in a year. The soil reaction is about pH 5·4–6·2, which favours the liberation of phosphate. It is of importance to note that the sub-soil was very well aerated, being a coarse sand in some cases. This means that iron salts must have existed largely in the ferric condition, and that toxic aluminium salts must have been absent or only present in minute amounts. With an equally acid clay soil their concentration would probably have been greater.

TABLE II.

Plant.	Situations observed.	Limits of occurrence, pH.	Notes.
Achillea Millefolium, . .	8	6·0–9·0	Mainly over pH 8.
Agrimonia Eupatoria, . .	2	6·8–8·2	
A. Eupatoria, var. *odorata* Mill,	2	5·4–6·4	
Alliaria officinalis, . .	2	7·0–7·4	
Alyssum maritimum, . .	2	7·6–8·2	Abundant where found.
Anagallis arvensis, . . .	7	7·2–8·2	
A. tenella, ...	1	5·4	Abundant.
Anthyllis vulneraria, . .	1	8·3	Do.
Arabis hirsuta, . . .	1	8·6–9·0	Scarce.
Arctium Lappa, . . .	1	5·4	
Arenaria peploides, . . .	2	8·0	Plentiful on sand and among shingle.
Armeria vulgaris, . .	3	6·6–8·2	Abundant at pH 6·6.
Bellis perennis, . . .	7	5·4–8·4	Abundant about pH 8.
Brassica campestris, . . .	1	7·6	
B. Sinapis, Vis., . . .	2	7·6–7·8	
Calluna vulgaris, . . .	6	4·6–5·8	Abundant at pH 4·6–5·2.
Capsella Bursa-pastoris, . .	4	6·8–7·8	
Cardamine pratensis, . .	4	6·8–7·6	Plentiful at pH 7·2.
Centranthus ruber, . . .	6	7·4–9·0	Abundant at pH 8·6–9·0.
Chrysanthemum Leucanthemum,	5	6·6–8·2	
Circaea lutetiana, . . .	1	7·2	
Cochlearia danica, . . .	4	7·6–7·8	Plentiful.
Convolvulus arvensis, . .	4	7·6–8·2	
C. sepium, ...	6	7·2–7·8	
C. Soldanella, ...	1	8·0	On dune sand.
Cotyledon Umbilicus, . .	8	5·4–7·8	Acid rocky banks and mortar of walls.
Crataegus Oxyacantha, . .	8	5·4–8·0	Only surface soil examined.
Crithmum maritimum, . .	3	7·5–8·2	Plentiful.
Digitalis purpurea. . .	2	5·4–7·2	Plentiful at pH 5·4, earthy soil.

TABLE II—*continued.*

Plant.	Situations observed.	Limits of occurrence, pH.	Notes.
Drosera rotundifolia, . . .	1	5·4	Plentiful.
Elymus arenarius, . .	1	8·0	Abundant on sand.
Epilobium parviflorum, . .	1	7·6	Plentiful.
Equisetum arvense, . .	1	8·0	
Erica cinerea, ...	6	4·6–6·0	Abundant pH 4·6–5·4, peaty soil or earthy.
E. Tetralix, ...	1	5·4	Abundant.
E. vagans, ...	1	6·2	In cultivation with *E. cinerea*.
Eriophorum polystachion, . .	1	5·4	Plentiful pH 5·4, soil rather peaty.
Eriophorum sp., ...	1	6·8	Possibly one species.
Erodium cicutarium, var. *hirtum* L'Hér.,	2	7·4–8·3	Plentiful.
E. moschatum, L. Hér., . .	1	8·0	
Euphorbia Helioscopia, .	2	7·0–9·0	Plentiful pH 8·6–9·0.
E. Paralias, ...	2	8·0–8·2	Plentiful on dunes.
E. Peplus, ...	2	7·0–7·6	Plentiful in field.
E. portlandica (E. segetalis), .	1	8·2	On sandy waste.
Foeniculum vulgare, . . .	1	8·6–9·0	
Fumaria officinalis, . . .	2	6·0–7·6	
Galium verum, ...	3	7·4–8·0	Plentiful.
Hypericum Androsaemum, . .	1	6·8	
Iris foetidissima, . .	1	6·2	Dry soil. Frequent on limestone soils, viz. pH 8.
I. Pseudacorus, ...	1	6·4	Plentiful in wet soil.
Jasione montana, ...	6	4·1–6·8	
Linaria Cymbalaria, . . .	7	6·6–8·0	Plentiful, banks and in mortar.
L. vulgaris, ...	1	8·2	
Lonicera Periclymenum, . .	3	5·8–6·2	
Lotus corniculatus, . .	6	7·2–8·4	Plentiful.
Lychnis diurna Sib., . .	7	6·1–7·4	
L. Flos-cuculi, ...	1	6·4	
L. vespertina Sib., . . .	1	7·6	
Lycopodium Selago, . . .	1	5·0	Earthy soil, Snowdon.

TABLE II—*continued.*

Plant.	Situations observed.	Limits of occurrence, pH.	Notes.
Malva sylvestris, . . .	3	7·6–8·4	
Mentha aquatica, . . .	1	7·6	Plentiful.
Narthecium ossifragum, . .	1	5·4	Plentiful, somewhat peaty soil.
Nasturtium officinale, . .	1	7·0	
Nepeta Glechoma, . . .	3	6·0–7·4	
Ononis arvensis, . . .	3	7·5–8·2	
Orchis sp., ...	1	6·4	
Papaver Rhoeas, . . .	1	7·6	
Parietaria officinalis, . .	2	7·6–8·2	Plentiful in mortar.
Peltigera canina, . . .	7	5·0–7·6	Plentiful even at the extremes.
Plantago Coronopus, . . .	7	7·0–8·4	
Polygonum aviculare, . .	2	7·6–7·8	
P. Persicaria, . . .	1	7·0	
Polytrichum commune, . .	1	5·0	Peaty soil.
Poterium Sanguisorba, . .	4	6·2–8·0	
Primula veris, . . .	3	6·7–7·4	
Prunella vulgaris, . . .	3	5·6–7·0	
Prunus spinosa, . . .	3	5·4–6·2	Earthy soil; surface only tested.
Psamma arenaria, Beau., . .	1	8·2	Abundant on dune.
Pteris aquilina, . . .	10	5·0–7·6	Abundant pH 5·4. Limit 5·0–6·4 save for one at 7·6, which, however, was an abundant occurrence.
Rhododendron sp., . . .	1	5·4	Surface soil only tested, but neighbourhood all acid.
Rubia peregrina, . . .	2	6·2	Plentiful.
Rubus fruticosus, . . .	17	5·4–8·4	Tendency to group around pH 6 and 8.
Salsola Kali, . . .	2	8·0–8·8	
Salvia Verbenaca, . . .	6	7·0–7·7	Abundant around pH 7·6.
Scabiosa arvensis, . . .	1	7·6	
Scilla nutans, Sm., . . .	4	5·0–7·6	Abundant around pH 5.
S. autumnalis, . . .	2	6·6–7·0	Abundant.

TABLE II—*continued.*

Plant.	Situations observed.	Limits of occurrence, pH.	Notes.
Sedum acre, . . .	5	7·0–8·0	Abundant.
S. anglicum, . . .	4	5·4–6·0	Abundant.
Senecio vulgaris, . . .	5	7·6–8·4	
Silene maritima, With., . .	7	6·0–7·8	Abundant at pH 6·6–6·8.
Spergularia rupestris, . .	3	6·8–8·2	Plentiful at pH 6·8.
S. rubra, ...	1	6·8	
Sphagnum sp., ...	1	5·4	Abundant.
Stellaria Holostea, . . .	3	6·7–7·3	
Symphytum officinale, . .	2	7·6–7·8	
Taraxacum officinale, . .	9	6·1–9·0	
Trifolium pratense, . . .	2	7·8–9·0	
T. repens, . . .	2	8·0–9·0	
Ulex europaeus, . . .	27	5·4–8·6	Abundant at pH 6·0–6·8.
Urtica dioica, . . .	5	5·4–7·8	Abundant about pH 7·8.
Vaccinium Myrtillus, . .	3	4·6–5·1	Plentiful.
Verbascum Thapsus, . .	3	7·6–9·0	Single plants.
Veronica agrestis, . . .	1	7·6	
V. arvensis, . . .	5	6·0–8·4	Plentiful about pH 8.
V. Chamaedrys, . . .	4	6·0–7·4	
Viola canina, . . .	5	5·4–6·7	
V. tricolor, . . .	1	7·8	

Garden cultivation of wild plants.

It is well known that certain of our native plants die out in gardens unless in soil similar to that in which they occur naturally. The writer is indebted to Mrs. E. W. Sexton for information regarding attempts to grow the following in ground behind the Marine Biological Laboratory at Plymouth. The soil is over limestone, and varies in reaction from about pH 7·6–8·2.

It was found that when transplanted the plants always died in the case of *Anagallis tenella*, *Calluna vulgaris*, *Drosera rotundifolia*, *Erica cinerea*, *Narthecium ossifragum*, *Scilla autumnalis*, *Sedum anglicum*, and *Vaccinium Myrtillus*. Elsewhere *E. cinerea* and *E. vagans* grew well in a garden at pH 6·2.

Among those which flourished were *Anthyllis vulneraria*, *Armeria vulgaris*, and *Sedum acre*. Reference to Table II shows that the pH value of the soil is correct for the latter, but incorrect for the former list.

Sedum album also flourished, but has not been found growing wild. This is sometimes called *S. anglicum* by florists, which serves to explain a puzzling instance in which *S. anglicum* was alleged to grow well in limestone gardens near Plymouth and Cork. The two were evidently confused, as the species of this genus often are (Praeger, 1921). It is stated in the Cybele Hibernica that *S. album* grows wild in several places near Cork. Due attention to the correct soil reaction will render the growth of wild flowers less precarious in captivity. Wherry (1918) has pointed out that, though most orchids require an acid soil, yet certain species, of Cypripedium for example, have slightly alkaline habitats.

The hydrogen ion concentration of natural waters in relation to plant distribution.

When considering the reaction of the soil it is natural to inquire into that of the water draining or springing from it, and the relation it bears to plant distribution. Mention has already been made of the existence of certain springs which contain sulphuric acid. These, however, are rather rare.

Birge and Juday (1911) made a detailed examination of the Wisconsin lakes, correlating the animal and vegetable life with the alkalinity of the water to phenol phthalein, or its acidity to the same indicator. This monograph studies the oxygen content of the water also, and is a mine of information. Chambers (1912), too, observed the seasonal changes in the alkalinity and oxygen content of the ponds in the Missouri Botanic Gardens according as the algae abstracted carbonic acid and evolved oxygen. The numerous researches on the hydrogen ion concentration of sea water and its relation to the photosynthetic activity of the algal plankton will be discussed elsewhere.

More recently Saunders (1921) has shown that the water from wells and of the town supply at Cambridge is very constantly at pH 7·1–7·2, the districts examined being chalk and gault. Running streams, however, lose carbon dioxide till the bicarbonate equilibrium with the gases of the air is

reached, values of 8·25–8·5 being obtained for them, and for clean large ponds. Attention was also drawn by Saunders to the effect of photosynthesis in raising the reaction of the water to as much as pH 9·0 in a small pond containing masses of *Spirogyra*.

The writer has examined a variety of natural waters, the reactions of which are tabulated below.

TABLE III.

Source.	Locality.	pH.	Notes.
R. Gandak, . .	Pusa, Bihar, . .	8·6	Approximate value from colour chart with cresol red and thymol blue. The soil around is at pH 8·6–9·0.
Tap, . . .	,, ,, . .	8·0–8·2	Purified river water.
,, . . .	Basingstoke, Hants, .	7·2	Deep well supply, hard water, chalky district.
,, . . .	Lowestoft, Suffolk, .	7·8	Approximate. Hard water. Gave pH 9·1 when boiled.
,, . . .	London, . . .	8·0	Approximate. Hard water.
Reservoir, . . .	Blagdon (Bristol), . .	7·8	
Tap, . . .	Cork, . . .	6·9	R. Lee water, filtered.
,, . . .	Youghal, Co. Cork, .	7·0	
,, . .	Plymouth, Devon, . .	6·8–7·0	Dartmoor water; soil about pH 5·4.
Rain, . . .	,, ,, .	5·9	Collected in copper rain-gauge between 3 p.m. and 9 p.m., after a wet morning in November. Air was accordingly dustless. Examined an hour after collecting. Soon rose to pH 6·2 and over owing to solution of glass.
Stream, . . .	Brixton, Devon, . .	8·2	
,, . . .	Compton, Devon, . .	8·0	Schalstein and Devonian slates area, gave pH 8·96 when boiled.
,, . . .	Youghal, Co. Cork, .	8·3	Soil around at pH 7·0; earthy soil on hillside pH 5·4.
Shallow wells, . .	,, ,, .	6·4–6·6	Partly supplying the stream. Insolation with algae raised this water to the neighbourhood of pH 9.
Shallow well, . .	Maker, Cornwall, . .	6·7	The Staddon grit-soil around is at pH 7–7·8.
Small reservoir, . .	Staddon Heights, Devon,	8·0	Or somewhat more. May.
,, ,, . .	,, ,, ,,	8·4	In full sunlight, October. The water was green, with green flagellates, abundant *Tetraëdron minimum* and *Scenedesmus obliquus*; when insolated in a bottle it rose to pH 9·7, denoting presence of magnesium salts.

TABLE III—*continued.*

Source.	Locality.	pH.	Notes.
Pipe from well supplying the reservoir,	Staddon Heights, Devon,	6·8	
Large pond, inlet, .	Blachford, Cornwood, Devon,	7·02	R. Yealm flows in and out of this pond; Dartmoor water; May.
Large pond, among weeds.	,, ,, ,, .	7·37	
Large pond, outlet, .	,, ,, ,, .	6·93	
Pond water, . .	,, ,, ,, .	6·80	In October. Gives pH 8·5 on boiling.
,, ,, . . .	,, ,, ,, .	6·40	In November. *Myriophyllum spicatum* grows here.
R. Yealm, . . .	,, ,, ,, .	6·58	November, at rapids below pond. Note increase in pH by aeration.
Leat, . . .	,, ,, ,, .	6·41	Leat water comes from Yealm, just above rapids. It supplies tank.
Trout tank, . .	,, ,, ,, .	6·42	
Small stream from pipe,	,, ,, ,, .	7·3	Flows into pond from west. On boiling, it reaches pH 8·9, and is therefore much richer in calcium carbonate than the river water.
Garden pond, . .	,, ,, ,, .	6·3	*Lemna minor* in abundance.
Small stream, . .	,, ,, ,, .	6·7	Flows into Yealm from east, above pond.
Small stream from pipe,	,, ,, ,, .	6·4	Flows into Yealm from east, below pond.
R. Piall, . . .	Slade, Cornwood, . .	6·83	In late October; flows into Yealm from west.
Spring, . . .	,, ,, . .	6·0	From pipe. Siliceous rocks of Culm measures; October.
,, . . .	,, ,, . .	6·7	From pipe. Different origin.
Small tank, . .	,, ,, . .	7·1	Supplied by second spring. In among weeds.
Loch Lannsaidh, .	Dornoch, Scotland, .	7·1	Gave pH 8·8 when boiled. November sample; poor in plankton; had *Gymnodinium sp.* (colourless Peridinian), common; green flagellate; a few diatoms *Asterionella sp.* and *Nitzschia sp.*; also a small, round green alga.

It should be noted that the Dartmoor drainage water of the R. Yealm is at a lower pH value in November than in May or October. This is probably due to the fact that in a rainy month it more nearly approximates to rain-water, and contains less calcium bicarbonate. The decay of leaves and the

less vigorous photosynthesis of the diminished plankton and water plants also tend to increase the amount of carbon dioxide; and, furthermore, the lower temperature of the water results in a large amount being retained in solution. These factors also tend to diminish the pH values, namely, to increase the hydrogen ion concentration.

From what has been said already it will at once be recognized that these results recorded in Table III point to the effect of the carbon dioxide from the air and from the soil gases in lowering the reaction that could be produced by the amount of calcium carbonate present. Even with upland water from Dartmoor taken from the R. Yealm at Blachford the acidity pH 6·8 is entirely due to excess of carbonic acid. On driving this off, by boiling in a carefully tested hard glass tube, as a maximum value pH 8·5 was reached. Assuming this to be due to calcium carbonate (viz., not to magnesium), it is possible to calculate the concentration of this salt. A saturated solution contains 0·0131 gram per litre, and gives as reaction pH 9·015 or C_{OH} $1{\cdot}05 \times 10^{-5}$. Now, pH 8·5 corresponds to C_{OH} $3{\cdot}2 \times 10^{-6}$; and therefore to 0·0040 gram per litre if it be assumed that at these dilutions the concentrations of the hydroxyl ions are proportional to the amounts of calcium carbonate.

The assumption may not be quite correct; for a more concentrated solution it certainly would be erroneous (see Clark, 1920, p. 29). To test this directly, a solution was prepared by boiling distilled water with calcite. In reality, the solution was not quite saturated, as it gave pH 8·96 instead of pH 9·01. This was diluted with the same distilled water, which when freshly boiled was at pH 7·1, as shown by brom thymol blue and phenol red, two volumes being added to one of calcite solution. When freshly boiled, this mixture was at pH 8·6 to both cresol red and thymol blue. Since it contains 0·004 gram per litre, the calculation showing that the solution at pH 8·5 contained 0·0040 gram must be tolerably correct. This method for finding the bicarbonate content of such soft waters is very rapid; its use will be treated in more detail elsewhere. The solution at pH 8·5 is of normality 4×10^{-5} with respect to calcium carbonate. This is just over three-tenths of the amount required for saturation in absence of carbon dioxide, but only about six per cent. of saturation when in equilibrium with the atmosphere. Photosynthesis in Blachford water cannot therefore produce a reaction more alkaline than pH 8·5 as an upper limit. This was tested with a small glass aquarium which had stood nearly four months with Blachford water and the plants growing naturally in it. In subdued light the tank was at pH 6·8. After an hour and a quarter in full October sunshine it had risen to pH 7·6, and after three and a half hours to pH 8·6. This is slightly above the limit reached by boiling the Blachford water direct, but the prolonged exposure

to the glass probably brought traces of alkali into solution. A duplicate with half Plymouth tap-water and half Blachford water also reached pH 8·6. A third vessel in good north light, and plentifully supplied with algae in Blachford water, was at pH 8·5; and a fourth which had stood for over a year, with added chalk, was at pH 8·95 when similarly illuminated. Since pure calcite, when boiled with water, only gives a reaction slightly over pH 9·0, this is near the limit. The value pH 9·7 given by the Staddon reservoir water, with its natural algal flora, is probably explicable on the assumption that small amounts of magnesium were also present, as is highly probable in a situation a quarter of a mile from the sea. Similar high values are found in sea-water during insolation with algae. Such photosynthetic changes have been studied in relation to their dynamics by Osterhout and Haas (1918).

Thus, both under its natural conditions and during intense insolation in jars, soft upland water, such as the Blachford and Plymouth water, is less alkaline than a water richer in the carbonates of the alkaline earths. The difference in the algal and general flora is probably correspondingly marked, though as yet it has not been examined in any detail. It is well known, however, that certain species are found mainly or exclusively in these soft waters, and the correlation of their occurrence and the pH value of the water will be of interest. Skene (1915) showed a striking relationship between the occurrence of various species of Sphagnum and their powers of growth in solutions of different normality, from N/250 to N/5000 acid or alkali. A study of the water in which they occur would probably demonstrate that the hydrogen ion concentration is of importance here also.

With natural waters of low bicarbonate content the buffer action is extremely slight, so great care must be taken in the preparation of the indicators, of which small quantities only should be used, so as to minimize error from the reaction of the latter. With solutions which are boiled the indicator should be added only after cooling again. Whenever possible, the use of two indicators is recommended, one with the middle region of its range above and one below the reaction of the liquid being tested. Rubber stoppers should also be kept away from water samples, since in contact with distilled water a reaction of pH 5·4 may be developed. Prolonged boiling of the rubber with changes of water minimizes this error. Special care should be taken to avoid contact with the ground-glass portion of stoppered bottles, especially those of white glass. Distilled water may become sensibly alkaline in a bottle; accordingly every bottle used should be tested.

Pure toluene has been found serviceable in preventing the growth of moulds in the Clark and Lubs standard buffer mixtures.

In conclusion, it must be stated that the experimental work recorded in this and the accompanying papers was carried out in the School of Botany, Trinity College, Dublin, and at the Marine Biological Laboratory, Plymouth. Determinations were also made at the Agricultural Research Institute, Pusa, India, and at various other places during field work. To the Directors of the laboratories mentioned the author wishes to record his indebtedness for the facilities afforded. The work was rendered possible by a grant from the Department of Scientific and Industrial Research, which covered the expenses of the special reagents. Thanks are also due to Mr. W. A. Davis, Pusa, for numerous samples of soil; to Dr. E. L. Fox, Plymouth, and Mr. C. A. Pode, Slade, for water samples and access to streams; also to Miss M. V. Lebour for some plankton identifications.

The bibliography is common to the three papers issued in this cover, and, with but few exceptions, references to be found in Clark's (1920) list have been omitted from it. They may be identified there by name and date given in the text.

Summary.

1. The theoretical maximum alkalinity due to calcium carbonate only is pH 9·01, which may be attained experimentally in the absence of carbon dioxide. The corresponding bicarbonate in equilibrium with the gases of the atmosphere is at pH 8·37 at 16° C., becoming more alkaline at higher temperatures. Owing to the high content of carbon dioxide in the soil, the pH values of limestone soils are usually lower, and vary with the aeration.

2. The theoretical maximum alkalinity for magnesium carbonate is pH 10·0. Dolomite soils may thus attain to greater alkalinity than limestone soils.

3. Alkalinity of over pH 10, due to sodium carbonate, may be reduced to pH 8 by the addition of calcium sulphate. The former reaction is injurious or destructive to vegetable cells, whilst the latter is favourable to most plants.

4. Soil acidity may be occasioned by the oxidation of sulphur from iron pyrites. This acidity favours the production of available phosphate, and is accordingly beneficial to certain plants.

5. Owing to production of carbonic acid by bacteria, a soil extract may decrease in alkalinity from pH 8·7 to 7·2, or less. The result in the soil appears to be to render iron salts more readily available in calcareous soil when inundated than when uncovered. The alteration is usually more rapid in soils from the top six inches than at greater depths.

6. Continuous manuring with sulphate of ammonium, or of potassium, decreases the effective soil alkalinity, even in a calcareous silt, but by a small amount only, about pH 0·2–0·4 in cases examined.

7. An acid soil extract is only slightly altered by boiling, from pH 5·4 to 5·7 in one instance. Alkaline extracts tend to reach the maximum value for calcium carbonate, pH 9·0. Higher values, such as pH 9·2, appear to indicate the presence of magnesium in small amount. The altered reaction is probably of importance in inhibiting the growth of certain soil organisms in heated soil.

8. When arranged in order of decreasing alkalinity, soils derived from different materials stand as follows :—Calcareous silt, limestone, sandstone, calcareous tuff (schalstein) with slate, slate, pillow lava (spilite), felsite, and granite. The values are modified in certain places, for proximity to the coast lessens acidity, and a high gradient often increases it. The results relate mainly to Devonian strata and accompanying volcanic or plutonic rocks.

9. Records are given for the hydrogen ion concentration of the habitats of over a hundred native plants. These show that this measurement is a valuable index of various soil conditions, and that many plants are limited to a short range of pH values. Others, with a wider range, occur mainly in one portion of it, but some plants grow well at widely different soil reactions. A distinction must be made between acid peaty soils, acid clay soils, and acid sandy soils.

10. The cultivation of wild flowers in a garden is dependent for its success upon the soil reaction of the natural habitats being maintained.

11. Natural waters, even from the peaty districts examined, contain no acid other than carbonic. Water in a spring may be at pH 6·4, and the stream flowing from it at pH 8·3, when in equilibrium with the atmospheric concentration of carbon dioxide. Photosynthesis may raise water containing magnesium salts to pH. 9·7. The method of limiting ionic concentrations may be applied to determine the amount of calcium carbonate in a soft water. This method shows Dartmoor water (R. Yealm) to contain 0·004 gram per litre of calcium carbonate in October. The hydrogen ion concentration of natural soft waters tends to increase during winter and to decrease in summer.

XXXI.

THE HYDROGEN ION CONCENTRATION OF PLANT CELLS.

By W. R. G. ATKINS, O.B.E., Sc.D., F.I.C.

[Communicated by Professor H. H. Dixon, Sc.D., F.R.S.]

[Read November 22, 1921. Published February 2, 1922.]

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Introduction.

An exact knowledge of the reaction of living cells is a necessity for the study of the chemical changes taking place in their metabolism. That this is so will be appreciated by a consideration of the influence of hydrogen ion concentration upon such changes as, for example, the molecular rearrangements of the sugars as studied by Nef (1911), or the rates of oxidation of sugars and of cystein (Mathews, 1909). In enzyme chemistry the controlling influence of the hydrogen ion concentration has been strikingly demonstrated by numerous researches, such as those of Sörensen (1909) upon invertase, catalase, and pepsin; of Michaelis (1909, 1914) upon invertase and malt diastase; of Bunzel (1916) and of Reed (1916) on oxidases; of Okada (1916) on the proteoclastic action of taka-diastase; of Frankel (1917) on papain; of Sherman, Thomas, and Baldwin (1919) upon three typical amylases; of Harvey (1920) on catalase in diseased leaves. Furthermore, the hydrogen ion concentration has been found to regulate the physical condition of proteins and similar substances; accounts of work in this line are given by Loeb (1918), Robertson (1920), MacDougal (1920), Cohn, Gross, and Johnson (1920), and Zoller (1921). For a full bibliography, that given by Clark (1920) may be consulted. It contains over eleven hundred references; and the list given by Schmidt and Hoagland (1919) has over four hundred and fifty.

No further emphasis need be laid upon the desirability of precise information on a factor so intimately concerned with the regulation of many processes of the cell.

As far back as 1902 Friedenthal applied the method of measuring electrical potential differences to the determination of the reaction of animal liquids. Its application to plant physiology came much later, though mentioned by Friedenthal (1910). But from 1916 onwards a number of determinations have been made, such as those of Haas (1916, '17, '19, '20); of Bunzel (1916); Reed (1916, 1 and 2); Hempel (1917); Kappen (1918); Patten and Mains, quoted by Clark and Lubs (1917); Truog and Meacham (1918); Fred and Davenport (1918); Clevenger (1919); Hoagland (1919); Swanson and Tague (1919). The results of some of these researches will be mentioned further on.

Hydrogen ion concentrations met with in plants.

From early work on the subject it was established that plant cells might be neutral or slightly acid to litmus, and the rather strong acidity of unripe fruits is well known. Hof (1909) used iodo-eosin as a test for free alkali in dried-up plant tissues; but results thus obtained are, it seems, highly misleading if taken to apply to the living plant.

The numerous electrometric and colorimetric researches already mentioned showed that plant-sap ranges from almost neutrality to pH 1·7, the value for lime juice, as given by Patten and Mains. Values for other fruits ranged from pH 4·5 downwards, viz. in the direction of greater acidity; but a lesser degree of acidity is met with in massive storage organs, such as potato tuber pH 6·2, sweet potato pH 5·7–6·6, beet pH 6·6. For those unaccustomed to think of acidity in pH values it may be of use to mention Wherry's (1919) suggested nomenclature for soil studies; this author takes the hydrogen ion concentration of pure water as unity. For this pH = 7; for a solution of acidity pH = 6, the "specific acidity" is 10, because the concentration in hydrogen ions is ten times as great as in pure water. Thus it may be seen that the "specific acidity" of lime juice, taking it as pH = 2, is as great as 100,000. It is 10,000 times more acid than potato juice. An even higher value, pH = 1·4, was found by the writer for an Indian berry, something like a gooseberry in appearance. For comparison it may be mentioned that the value for a centinormal solution of hydrochloric acid is pH = 2; yet this is usually considered a very dilute acid. For adult gastric juice pH = 1·8 approximately, and a solution of carbon dioxide in water nearly saturated at 25° C. is at pH = 4·8. On the other side the

pH value for sea-water is about 8·2; it is, therefore, slightly alkaline, or, in Wherry's terms, it has a specific alkalinity of over 10.

As far as the writer is aware, but few plant-saps are alkaline, and those only very slightly so, as, for example, the roots of wheat, as found by Kappen (1918). Haas (1916) has drawn attention to the fact that the sap may in some cases lie between pH 7 and 8. The writer has found some marine algae to be alkaline pH 7·3 or slightly less. Colorimetric work may, where clear sap solutions are available, be brought to much precision by the use of a Kober (1917) colorimeter, as pointed out by Duggar and Dodge (1919). The writer has found the Duboscq colorimeter very satisfactory for the purpose also. All researches based on electrometric or colorimetric estimations on expressed sap are open to possible sources of error in the changes undergone during extraction. For example, as shown by Mason (1920), leaves exposed to toluene vapour, in order to increase the permeability of the cells and to render the expression of the sap easier, are found to develop a considerable amount of heat owing to rapid oxidations. The well-known browning of many plant juices when expressed shows the influence of oxidation, which may alter the acidity. McClendon and Sharp (1919) record a change in carrot juice from pH 5·85 to 5·73 on standing twenty minutes in air. For this reason the work of Haas (1916) is of special interest, inasmuch as he uses the anthocyan pigments of petals as natural indicators of reaction in the living cell. It has frequently been assumed that a red colour denotes an acid cell-sap in the petal, and a blue an alkaline. Haas, however, extracted the pigments, and observed the colour given when in solutions of known pH. In this manner he showed that, for example, the pansy, which is blue, yields an anthocyanin which is rose from pH 1 to pH 4, blue at pH 5, and blue-green from pH 6–8. From pH 9–11 it is green, and finally yellow at pH 12. It is clear, therefore, that the living cells are in the neighbourhood of pH 5. It is accordingly not alkaline, but of well-marked acidity. The fading of colour in the cells as they die is accompanied by a decrease in acidity.

Determinations involving small quantities of liquid.

When only small quantities of liquid are available it is often allowable to dilute the solution sufficiently for the pH to be determined with very approximate accuracy. As pointed out by Clark (1920), under certain conditions the effect of dilution is almost negligible. This device may be employed with advantage for highly coloured or turbid solutions.

Haas (1919) made use of the comparison of drops of solution on white porcelain against drops of standard solution, with addition of a suitable

indicator, and of specially prepared indicator papers. Wagner (1916) previously employed the drop method, using lacmosol as indicator in work on plant immunity. Indicator papers were also used by Hempel (1917) in a research on succulent plants.

Felton (1921) has found this drop method very serviceable for work on tissue cultures, and thinks it preferable to working with 2–3 mm. tubes and diluted liquid, owing to dilution errors in fluids of low buffer action. It was also tried by Jameson and Atkins (1921) for work on the tissues of the silk-worm, and has been used by the writer for a year with, it is thought, fairly reliable results.

Determinations of hydrogen ion concentrations in plant tissues.

When working upon the hydrogen ion changes taking place in the course of the *mahai* or fermentation of indigo, it seemed desirable to see what relation the final stage (which was at about pH 5·6, as far as could be judged in the brownish liquid of a small-scale fermentation) bore to the acidity of the indigo leaf. It may be remarked that the initial value was about pH 8·5, i.e., that of the river-water. The plan adopted was to crush portions of indigo leaf on white porcelain, with the addition of one or two drops of distilled water, and to compare the colours given with methyl red and brom cresol purple against those which appeared with the standard buffer solutions. It is always advisable to use two indicators where possible, as it lessens the chance of any serious error. In this way it was found that the leaves of young and old plants were of acidity pH 5·6, whereas cotyledons still in the seed-coat were slightly less acid, pH 5·8. Determinations were thus made upon various Indian plants, and are recorded elsewhere (Atkins 1921). In addition to working with crushed tissues, an attempt was made to use the indicator on sections in order to see whether adjacent cells differed noticeably in acidity, for it seemed possible that in crushing a stem the result might give a very misleading idea of the true condition of the conducting tracts.

For example, it was found that varieties of the castor-oil plant (*Ricinus communis*), grown at Pusa, gave pH 4·6 for the stem, for both red- and green-stemmed plants, but the tissues were not examined separately. It was similarly found that young leaves, older leaves, and a young flower were at pH 4·8. A section across the seed-capsule with very immature seeds showed, however, that the wall tissues were at pH 4·8, while the seeds were much less acid, pH 5·4. Had the capsule been crushed, an erroneous result would have been obtained. The acidity of the crushed pollen sacs was also pH 5·4, as nearly as could be judged.

These values are at least comparable ones, though on account of the presence of proteins in the cells an error is introduced. The indicators used, however, were selected by Clark and Lubs (1917) as having small errors from this source, as has been further demonstrated by Homer (1917) in serum studies.

The application of the method to the mapping out of the acidity of tissues may be illustrated by the following :—

The Labiate *Salvia Verbenaca* grows plentifully near the Plymouth laboratory, and affords convenient material. A fresh transverse section of the stem was stained with various indicators, and it was seen that di-ethyl red (Lubs and Clark, 1915) gave with the pith a yellowish colour, which was also seen in the medullary rays and cambium. The wood was a good red in the walls ; the bast fibres were deep red in the cells, as were also the cells of the sclerenchyma in the four angles of the stem. The cortical parenchyma between the bast fibres and assimilating cells was yellowish ; the assimilating cells were apparently a very light yellow, as far as could be judged in the presence of the chloroplasts. The epidermis was seen to be a deep red, but this is its natural colour; to avoid error it is always necessary to examine an unstained section.

Since di-ethyl red changes from red to yellow between pH 5·8 and 6·0, it is at once clear that the red-stained portions must be at least as acid as pH 5·8, and the yellow equal to or less acid than pH 6·0.

Using methyl red, the sclerenchyma and bast fibres give a salmon pink to pink colour, corresponding to pH 5·4 to 5·2 ; the wood appears a faint pink, not more acid than pH 5·4 to 5·6, and the medullary rays are yellow. The upper limits of acidity are therefore fixed.

It remains to determine the acidity of the portions appearing uniformly yellow of the same tint with di-ethyl red. With phenol red the full yellow colour appeared, showing an acidity of pH 6·6 or more. With brom thymol blue a yellow with a green tinge was seen, matching the colour with pH 6·2. A green tinge in the tissue may, however, make this acidity slightly too low. Accordingly a small cube of the medulla was excised with a stainless steel knife, which was found very useful for this work, and was crushed in a watch-glass with an agate pestle, and to this one drop of the dilute brom cresol purple was added. The tint was matched by taking two drops of standard buffer solutions, pH 5·8, 6·0, and 6·2, with one drop of the reagent, since the volume of the crushed tissue was closely the same as that of two drops. The colours were pH 5·8 dirty greenish, 6·0 slaty blue, and 6·2 deep slate blue. The medullary tissue was a very close match to 6·0.

Thus the various tissues seen in the cross-section of this stem with the

aid of a hand lens or low-power (two-thirds inch) objective have been mapped out with tolerable accuracy as lying betwen pH 5·2 and 6·0. The results may be summed up as follows, the C_H values corresponding to the pH values being also given, to emphasize the magnitude of the acidity differences involved:—

	pH	C_H
Sclerenchyma and bast fibres, . .	5·2–5·4	$0{\cdot}63\text{–}0{\cdot}40 \times 10^{-5}$
Wood walls, ...	5·4–5·6	0·40–0·25 „
Medullary rays, medullary and cortical parenchyma, ...	6·0	0·10 „

Thus it is seen that the acidity in the sclerenchyma is four to six times as great as in the adjacent cortical cells, but, on account of the relatively great bulk of the parenchyma, expressed sap would appear to be more nearly of the acidity given by the latter. This relatively high acidity of the wood and sclerenchyma seems to be quite a normal occurrence, and cannot fail to have a physiological significance.

As a further example of the difference in reaction between portions of a plant in fairly close proximity may be mentioned the behaviour of wheat seedlings (Pusa 4 variety). These were removed from soil with a strongly alkaline reaction, pH 8·8, and the roots were washed till the washing water gave pH 7. The roots then were at pH 6·8, as shown by brom thymol blue and phenol red. The white leaf bases and the young green leaves were both at pH 5·4, as shown by methyl red and brom cresol purple. As already mentioned, Kappen (1918) found cereal roots neutral, those of wheat even being slightly alkaline. At Pusa the roots were slightly on the acid side of neutrality for wheat, an identical value being given by those of both oats and rice, viz. pH 6·8. The leaves of the oats were at pH 5·4, while with the rice the leaf gave pH 4·8, the first and third internodes of the stem being at pH 5·0, intermediate with the value of the roots.

With a very simple outfit it is possible to make determinations of the acidity of plant tissues in the field. This is of value in work correlating the acidity of the plant and the soil, and in cases where for any reason a laboratory is not to hand. A few small tubes of the necessary indicators are required, one or more glass rods, and a few watch glasses. It is also well to have a knife of stainless steel, a hand lens, and a bottle of distilled water, or of rain water. On such expeditions a separate reprint of the coloured chart of indicators at various pH values given by Clark is very useful, and the buffer tablets already mentioned are a desirable item, and add little to

the weight. It is necessary to test the bottle used for water to make sure that it does not give off an appreciable amount of alkali during the course of the work. It is found that the ground-glass region of the stopper is specially liable to cause trouble of this sort. It is, therefore, preferable to remove water with a pipette and rubber teat rather than to pour it out. With a little experience as to the tints given by the indicators it is possible to work with approximate accuracy of pH 0·2 to 0·4 with the aid of the chart. As a rule the determinations will be accurate to pH 0·2 or less, especially if use is made of two or more indicators. If buffer tablets are carried, the work can be as precise as in a laboratory. As an example of such determinations, the following may be mentioned:—

Anagallis arvensis, transverse and longitudinal sections of the stem stained red in the vascular bundles and yellow in the pith with methyl red. The former was, therefore, more acid than pH 5·2, and the latter less acid. Di-ethyl red was not available at the time, so the lower limit was not determined, but it was at least pH 5·8.

Taraxacum officinale, transverse section of stem showed pH 4·6; latex abundant; rootstock latex canals pH 4·4–4·8; vascular bundles much the same, but not quite as acid as the latex, which gave a more purple tint with methyl red; rootstock medulla pH 5·4. Leaf parenchyma lay close to pH 5·8, as shown by brom cresol purple, and by its yellow colour to methyl red, whereas the midrib was about pH 4·6.

Cochlearia armoracia, transverse section of stem was at pH 4·6 in vascular bundles and sclerenchyma, pH 6·0, or slightly less acid, in parenchyma. The leaf parenchyma was at pH 5·4.

These three plants were growing in soil which gave with water pH 7·6–7·2, as shown by phenol red.

In conclusion it may be mentioned that in cases in which the sap is coloured, or the crushed material is turbid or milky, it is sometimes useful, having made an approximate determination of the pH value with indicator and drops of standard buffer solution, to try adding the buffer solution of the nearest pH value to a portion of the crushed tissue. Since the buffer action of the plant sap, whilst by no means negligible, as shown by Hempel (1917), is not at all as great as that of the added solution, the alteration of the pH value of the latter can be but slight with sap of approximately the same pH value. The resulting colour of the true pH is, however, modified by the tint or turbidity of the tissue, and thus a good match may be obtained with the plant sap. This is especially useful with dichroic indicators, such as brom phenol blue and brom cresol purple.

Hydrogen ion concentrations of plant secretions and exudations.

It seemed of interest to study the reaction of the transpiration stream, since, owing to the fact that it is evaporated in the leaves, the acidity, if any, would be concentrated in course of time. The determination is not easily made, since even if a woody stem is cut and centrifuged (Dixon and Atkins, 1915) there is the risk of contamination with the sap of living cells, and this, though diluted, might cause an appreciable error. It is hoped, however, to test this by direct experiment, and to see whether the variation in sugars previously shown is accompanied by any change in acidity.

Recourse was had to the liquid which drips from the leaf tips of *Colocasia antiquorum*. Attention had been drawn to the great purity of this water by early workers, Duchartre (1859) and Musset (1865), and a plant growing in the Trinity College, Dublin, Botanic Gardens was found by Dixon (1914) to have a freezing point indistinguishable from pure water when tested by the thermoelectric method, one couple being in each of the two liquids. Further, the electrical conductivity was less than that of Dublin tap-water, a very soft upland water from a granite and quartzite area. The solids amounted only to 0·012 per cent. as determined by evaporating 20 c.c. of the liquid. It had also been shown by Miss Flood (1919) that this liquid is in direct connexion with the conducting tracts of the leaf, and has therefore passed through living cells only in the roots. The colorimetric determination with brom thymol blue, using 10 c.c. of the liquid which issues from the leaf tips, gave pH 6·8, which is a value given by distilled water quite frequently owing to the absorption of carbon dioxide. With the same indicator it was found that sap pressed from the leaf stalk was at pH 5·4, and the leaf tissue at 6·0. It is clear, therefore, that this water of the transpiration stream is entirely uncontaminated with acids, though it has passed through the root tissues and the conducting tracts of the entire plant.

Another liquid of interest is the water found in the axils of the leaves of *Dipsacus laciniatus*. This has been shown by L. B. Smyth (private communication) to be without digestive action. A plant growing in the Botanic Gardens was found to contain much water in the axils. This was at pH 6·8 in a lower leaf and 7·0 in an upper one. The lower one contained dead flies. Since the water was present after a considerable period of drought, it is clearly derived from the plant, and is quite possibly in connexion with the conducting system. This point was not, however, investigated.

The pitchers of *Sarracenia* spp. are also well known to contain liquid. It is stated that this is without digestive action, but that insects caught in it

are decomposed by bacteria and the products absorbed. It, therefore, seemed probable that this water would be nearly neutral in reaction. Pitchers of *Sarracenia purpurea* × Hort., in the Botanic Gardens, were examined, and an old one was found to give a red colour with phenol red, denoting an alkaline reaction. This, however, may have been due to chance contamination, such as from a flake of whiting from the windows, for it was found that young pitchers contained a clear colourless liquid of pH 5·0 to 5·2, as shown by methyl red. Older pitchers of *S. tolliana* × Hort., varied from pH 6·0 to 6·8 when tested with brom thymol blue. It was subsequently discovered that Wherry (1920) had tested this liquid in several species growing wild in the swamps of the middle Atlantic States of the U.S.A. He found that the liquid in the pitchers was usually "mediacid" or "subacid," namely, pH 4 to 5 and pH 5 to 6 respectively. He attributed the acidity chiefly to carbonic acid, but, though a saturated solution is in the mediacid region, it would soon lose carbon dioxide, so another acid must be present. Where the reaction was found to be between pH 6 and 8, he considers that calcium bicarbonate had dropped into the upturned pitchers from leaves of other plants which drew from alkaline water supplies. The *Sarracenia* plants were always on acid hummocks when the soil around was alkaline. It has, however, been shown by Hepburn (1918) that the unopened pitchers of *Sarracenia* are sterile, and the liquid has a proteolytic enzyme; in older opened pitchers bacteria are symbiotic. It is, therefore, not at all surprising that the contents of the pitchers should be acid when freshly opened, and it may be that the alkaline reaction found in some, both by Wherry and the writer, is not due to chance contamination, but to the products of digestion, or to those of bacterial action. Bodine (1921) has proved that protozoan cultures in hay or soil infusions change in time from an acid to an alkaline reaction. Possibly the old pitchers alter owing to the development of protozoa. This could be tested directly.

An attempt was made to examine the liquid in pitchers of *Nepenthes sp.*, as this is known to have a digestive action, but unfortunately the pitchers were dried up. *Drosera binata*, Labill., was, however, plentiful, and the leaf-hairs were well covered with the characteristic beads. On touching with a glass rod and stirring with water on a watch-glass it was observed that it was hard to get these mucilaginous droplets to mix. With brom thymol blue and phenol red the reaction given was pH 7·0, but the former indicator showed pH 6·4 when tested with the liquid round a dead fly. Thus it appears that acid may be secreted only when the hair is stimulated, or it may have come from the fly.

D. rotundifolia was obtained growing on Dartmoor, and on washing the glandular leaf-hairs with a drop of water they were found to be quite strongly acid, pH 4·8, or slightly more acid using methyl red. The crushed leaf was carefully compared with standards, and agreed with pH 3·0, using brom phenol blue. This was confirmed with thymol blue. The significance of this in connexion with enzyme action will be mentioned later.

Relation of the natural acidity of plant tissues to the activity of their enzymes.

Determinations made on the fruit of *Carica papaya* at Pusa showed that the latex of the skin of the unripe fruit was as acid as pH 5·4, an identical value being given by the hard central tissue. On looking up Frankel's (1917) work on purified commercial papain, it was seen that this enzyme works most rapidly at pH 5 when the temperature is 37° C. Taking this rate as 100, the rates from pH 3 to pH 7 are respectively 27, 91, 100, 63, 47.

The natural reaction of the fruit is, therefore, close to the optimal value for papain action. According to Kilmer (quoted from Mendel and Blood, 1910), the ripe fruit gives no latex when the skin is cut, and the pulp shows very little proteolytic activity. Unfortunately no ripe fruits were available for the examination of pH values. The marked action of hydrogen cyanide in activating papain, especially as regards the progress of the action up to the formation of amino-acids, as described by Mendel and Blood, may, perhaps, be of significance in the physiology of plants having cyanophoric glucosides.

Again, the recent work of Falk and M'Guire (1921) has shown that the action of soluble sucrase from bananas is most active at pH 3·5 to 4·5. Taking the activity at pH 4·0 as 100, the series at intervals of pH 0·5, from pH 3·0–6·5, is as follows :—37, 98, 100, 98, 94, 78, 27, 11, the rates being measured at 35° C. In this case also the reaction of the fruit lies in the neighbourhood of the optimum for the enzyme, a freshly picked small unripe fruit being of acidity pH = 4·6–4·8, that of the skin being 4·6 approximately. For the leaf-stalk a value pH 4·9 was found. The pulp and skin of a fully ripe banana, examined in England, were found to lie between pH 5·6 and pH 6·0 in different cells. In considering the optimum pH value for any enzyme, account must be taken of the temperature, for Compton (1921) has shown that the optimum value varies with the latter. Thus, for the maltase of *Aspergillus oryzae*, he found that for pH 3·0 the maximum hydrolysis occurred at 35·5° C., whereas at pH 7·2 it was not reached till 47° C. In the natural conditions of growth of the papaw and banana at Pusa, an air temperature in the shade of 35°–38° is quite normal, and in full sunlight the fruits, in spite of surface evaporation, must often attain considerably greater temperatures. When one considers that the action of maltase at

pH 3 has fallen from its maximum rate at 35·5° C. to about two-thirds of this rate at 39° C., and to roughly one-third at 42° C., the importance of high temperatures combined with high acidity is made clear. With lower acidity, however, the optimum has not been reached at 42° C. It is thus very natural that the reaction found for the plant tissue should be rather below the optimum acidity for 35·5° C., since the fruit is certainly exposed during its development to higher temperatures at which a degree of acidity optimal for 35·5° C. would have serious destructive action on the enzyme. In tropical climates the black bulb thermometer may reach 72° C.; and it has been found that thermometers placed inside small wooden frames about a foot square and two inches deep, covered with linen, aeroplane dope, and varnishes of various colours, quite commonly reach 50°–60° in sunlight. These measurements were not made to investigate fruit temperatures, but they serve to show how high are the temperatures that may be found under such atmospheric conditions. It accordingly seems reasonable to suppose that temperatures of 45°–50° may be met with in ripening fruit in the tropics. In England during full sunshine in July it was found that with an air temperature 25·1° C. an untreated mercury thermometer rose to 33·7 when insolated; and a similar thermometer inserted in a ripe banana reached 33·4°. The fruit is thus over 8° above air temperature; and in view of the fact that in Upper Egypt and the Sudan temperatures of 44° are common, it is unlikely that 50° is at all too high an estimate for the temperature to which fruits in tropical climates may be exposed. In this connexion the decrease in acidity of fruits as they ripen is to be noted, and as the ripening usually takes place in the hot weather, it is possibly advantageous for enzyme action that the initial higher degree of acidity should be reduced.

As previously mentioned, immature castor-oil seeds were found to give a reaction of pH 5·4, though the leaves were at pH 4·8. No details, quite parallel with those on papain, of the effect of various pH values on lipase action are to hand; but Armstrong and Gosney (1913, 1914) have shown that after the enzyme has been liberated from its zymogen by dilute acetic acid, the low acidity of oleic acid is most favourable to its action. A table given by these workers shows the action of 0·5 gram of castor bean lipase upon a solution of ethyl succinate according as increasing amounts of various acids were present. The percentage hydrolysed in twenty hours was 56·5 with no added acid, as against 60·5, 61·8, and 57·3 with N/40, N/20, and N/10 butyric acid respectively, and 8·1 per cent. with N/40 sulphuric acid. If one neglects the quite possibly considerable effect of the enzyme preparation in diminishing the hydrogen ion concentration, though acid itself, and determines the pH values corresponding to the above concentrations, it is found that the butyric acid values are pH 2·67, 2·85, and 2·93, arrived at by

interpolation between standard solutions at 2·60, 2·75, and 3·0 with thymol blue. For the sulphuric acid pH 1·7 was obtained. Thus, at the temperature of the laboratory this degree of acidity had a very destructive action on the enzyme. As pointed out, the pH values recorded here can only be upper limits for the acidity actually in contact with the enzyme. In this connexion the work of Falk (1915, 1917) is also of interest. He found lipolytic enzyme from castor beans could be separated into an albumin-like body more active towards ethyl butyrate than to glyceryl triacetate, and a globulin-like body more active to the latter. It was ascertained that after standing for a day at 0° C., in a liquid of acidity pH 3·5, the esterase had almost become inactive when tested subsequently by its hydrolytic activity at 38° C. At pH 4·5 one-third of its activity had been lost. The destruction of the lipase was not quite as great. It was further proved that after standing for a day at 0° at pH 8 the esterase was slightly less active than at pH 7, but at pH 11 it had lost 90 per cent. of its activity.

Thus it is evident that the destructive action of an acidity of pH 4·5 at 0° upon lipase must stand in close relation to the fact that in the seed the reaction of the stem and leaf, namely pH 4·6, is found to be reduced to pH 5·4, a change of C_H $0{\cdot}32 \times 10^{-4}$ to C_H $0{\cdot}04 \times 10^{-4}$, namely to one-eighth.

Indicators for use with plant tissues.

Of the very numerous substances which act as indicators, Sörensen (1909) selected a limited number as giving well-defined brilliant colours, and being relatively uninfluenced by salt and protein errors, whereby a discrepancy arises between the true hydrogen ion concentration as shown by the potential difference method and the colorimetic method. Sörensen excluded cochineal, Congo red, alizarin, and all litmus preparations. He included neutral red, which is remarkably free from the errors, and is of great value by reason of its penetration of the living cell without toxic effects when in moderate concentration, for which purpose it was originally introduced by Ehrlich. This indicator gives a good clear red from pH 7·0 to 6·6, and the more acid region. It is reddish from 7·2 to 7·6, and orange beyond that. The stain is of special use in testing the reaction of algal cells in an alkaline medium, as will be described in detail elsewhere.

Clark and Lubs (1915, 1917) introduced the phenol sulphone phthaleins for use as indicators, and selected the best. All those shown in Clark's list are of value, and by reason of their stability and ready solubility in water as sodium salts, as well as their brilliancy, they are to be preferred to some on Sörensen's list. They include methyl red, introduced by Palitzsch (1911), which, though not readily soluble, covers a range much used. Clark and Lubs aimed at reducing the number of indicators, and this has naturally

many advantages, when dealing with clear solutions especially. However, with plant sections and drops of liquid a somewhat larger selection of indicators is very useful. For example, though the range from pH 5·4 to 6·0 is covered both by methyl red and brom cresol purple, yet for the greater portion the tints are yellowish. This is not readily observed in tissues, hence the value of di-ethyl red introduced and found reliable by Lubs and Clark (1915), but excluded later as unnecessary. This changes from yellow at pH 6·0 to red at 5·8, the colour increasing in depth with increase of acidity. Furthermore, it is readily taken up by those plant tissues with which it gives a red colour, whereas brom cresol purple is not well retained. On the more acid side the useful range of methyl red ends at pH 4·4, at which point brom phenol blue is blue. Methyl orange is often of service for greater acidity, especially as brom phenol blue is dichroic. The former is yellow at pH 4·6, and varying shades of orange to 4·0; at 3·8 and 3·6 the red predominates, and beyond 3·4 it is clear red. This is just the region in which the dichroic blue has become a yellow, and so difficult to observe in a section. The colours and ranges mentioned apply only to the dilutions found suitable for colorimetric work.

Summary.

1. Plant cells are rarely alkaline, and pH 8 is not surpassed in them. On the acid side pH 1·4 has been observed.

2. By a microchemical method it is possible to determine the pH values of the cells and tissues. It has been found that the xylem is more acid than the pith and medullary rays, and the midrib of a leaf more acid than the parenchyma. Parenchymatous tissue is often in the neighbourhood of pH 6; woody tissue nearer pH 5, or more acid. When grown in neutral or alkaline soil, the root is usually less acid than the other portions of the plant. The influence of soil reaction is reserved for consideration in another paper.

3. The transpiration stream in *Colocasia antiquorum* is almost neutral, as is also the liquid in the leaf-base troughs of *Dipsacus laciniatus*. The pitchers of *Sarracenia spp.* may be as acid as pH 5, and the glandular secretion of *Drosera rotundifolia* may be even more acid.

4. It has been pointed out that the pH value met with in a tissue is usually near, but slightly less than, the optimum for the activity of the characteristic enzyme at ordinary air temperature. This ensures that the acidity does not destroy the enzyme at such higher temperatures as may be experienced by the plant under natural conditions.

5. Attention is drawn to the usefulness of di-ethyl red as a reagent for microchemical work, as it gives a red in a region where methyl red and brom cresol purple are yellowish; in this range many plant tissues are found to lie.

XXXII.

NOTE ON THE OCCURRENCE OF THE FINGER AND TOE DISEASE OF TURNIPS IN RELATION TO THE HYDROGEN ION CONCENTRATION OF THE SOIL.

By W. R. G. ATKINS, O.B.E., Sc.D.

[Communicated by Professor H. H. Dixon, Sc.D., F.R.S.]

[Read November 22, 1921. Published February 2, 1922.]

It has long been known that the disease occurs in soils poor in calcium salts. Hall (1910) has collated the results obtained by Voelcker and other workers, which show that finger and toe was found on turnips growing in soil containing calcium, expressed as oxide, to the amounts of 0·14, 0·08, 0·13, 0·31, 0·39 per cent. in five cases studied, whereas spots, some in the same fields, with 0·89, 0·52, and 0·43 per cent. were free from it.

The writer is indebted to Mr. T. Sherrard, Maryborough, Douglas, Cork, for the notes on the behaviour of two fields and for soil samples.

1. "This sample is infested very badly with finger and toe. No turnip or cabbage can grow in this soil."

2. "This sample is from the same land, only a stream of water dividing the fields. No trace of finger and toe is found here. It is absolutely free from fungus, and grows fine turnips."

Sieving showed that in each pebbles, &c., which did not pass 30 mesh to the inch, amounted to 23 per cent. of the air-dry soil. Of the good field, 65, and of the bad, 64 per cent. passed the 100-mesh sieve. The soil which passed the latter was found to contain 0·17 and 0·40 per cent. of lime in samples Nos. 1 and 2 respectively. In view of previous work, it is safe to attribute the behaviour of the two fields to their difference in calcium salts. The good field is, however, rather dangerously depleted, as Hall records disease in sandy soil with 0·31 per cent. of calcium oxide, and in clay soil with 0·39. The addition of lime or limestone to the bad field is clearly indicated, and it would probably benefit the other also.

The acidity of these soils was determined by the colorimetric method, as described and explained in a previous paper (Atkins, 1921). It was observed that the reaction was only very slightly different, the good field being less acid by an amount estimated at pH 0·1, using brom thymol blue and brom cresol purple as indicators. With neither was there

any doubt that the soil of the bad field was the more acid. Using brom thymol blue and the Clark and Lubs standard buffer solutions, which are at pH 0·2 intervals, it was seen that No. 1 was at pH 6·6, and No. 2 at pH 6·7, or slightly more, not up to 6·8. The difference appeared somewhat less with brom cresol purple. The actual numerical concentrations of hydrogen ion are respectively C_H 0·25 and 0·20 × 10^{-6} gram per litre.

It would be of interest to study a large number of samples, and it is hoped that this may draw attention to the value of the method, since, once the standards are prepared, it is very rapid, and comparisons can be made even without standards. Neutrality at 16° C. is at pH 7·1, and it is probably safe to conclude that a soil giving pH 6·9 or 7·0 will not be liable to have its turnip crop attacked, unless possibly when subjected to heavy infection through manure from animals fed on diseased turnips. It may be mentioned that cabbage as well as turnips failed completely on the No. 1 soil, though basic slag was used with both in successive years. This soil will grow nothing if touched in wet weather; a crop of mangolds was lost in trying to work it when wet. It gets sticky and then hardens, when nothing will grow. It seems that the texture of this clay soil would also be improved by liming, as the colloidal particles would be clumped thereby.

It would be of interest to make a biochemical examination of diseased and healthy plants to try to ascertain how it comes about that a deficiency in calcium salts renders the plant more susceptible. Possibly the effect is, in part at least, upon the fungus, which refuses to grow in a neutral or slightly alkaline medium. This statement, quoted from Hall (1910), is based on work done prior to the introduction of the present methods of determining hydrogen ion concentrations, so it would be of interest to ascertain the limits between which the fungus can be grown.

No direct proof has been given that the pathogen was present in the No. 2 field, and the conclusion put forward has been criticized on this score. In view of Hall's work, however, it seems to the writer that there is at least a reasonable degree of probability in favour of the presence of the pathogen, and further work will show to what extent the pH limits given here must be modified.

Summary.

One of two adjacent fields of similar clay soil was found to be badly infested with, and the other free from finger and toe disease in the turnip crop. It was found that they contained, respectively, 0·17 and 0·40 per cent. of calcium, calculated as oxide. The hydrogen ion concentration of the samples was pH 6·6 and pH 6·7, or slightly over, respectively, as determined colorimetrically, or, stated otherwise, C_H 0·25 and 0·20 × 10^{-6} grams per litre respectively.

BIBLIOGRAPHY.[1]

ALWAY, F. J., 1920.—1. Agricultural value and reclamation of Minnesota peat soils. Univ. of Minnesota Agr. Expt. Sta. Bull. 188, 1920. 2. Report of Golden Valley peat experimental fields. *Loc. cit.*, Bull. 194.

AMES, J. W., and BOLTZ, G. E., 1916.—Sulfur in relation to soils and crops. Ohio Agr. Expt. Sta. Bull. 292, p. 221.

AMES, J. W., and RICHMOND, T. E., 1918.—Effect of sulfofication and nitrification on rock phosphate. Soil Sci., **6**, 351.

ANGELSTEIN, U., 1911.—Über die Kohlensaureassimilation submerser Wasserpflanzen in Bikarbonat-und Karbonatlosungen. Beit. z. Biol. d. Pflanzen, **10**, 87–117.

ARMSTRONG, H. E., and GOSNEY, H. W., 1913, 1914.—Studies on enzyme action. Lipase 3 and 4. Proc. R. Soc., **86B**, 586, and **88B**, 176.

ARRHENIUS, O., 1920.—Öcologische Studien in den Stockholmer Schären. Stockholm.

ASTON, B. C., 1916.—Lime and magnesia in New Zealand soils. J. Agr. N.Z., **12**, 47, and Expt. Sta. Rec., **35**, 715.

ATKINS, W. R. G., 1921.—The hydrogen ion concentration of some Indian soils and plant juices. Imp. Dept. of Agric., India. Pusa Series.

ATKINS, W. R. G., 1921.—Relation of hydrogen ion concentration of the soil to plant distribution. *Nature*, Sept. 15, **108**, 80.

BAYLISS, W. M., 1915.—Principles of general physiology. London.

BEANS, H. T., and OAKES, E. T., 1920.—The determination of the hydrogen ion concentration in pure water by a method for measuring the electro-motive force of concentration cells of high internal resistance. J. Amer. Chem. Soc., 1920, **42**, 2116.

BJERRUM, N., and GJALDBAEK, J. K., 1919.—Examination of the factors which determine the reaction of the soil. Kong. Vet.-og. Landbohöjskole Aarskr., 48–91.

BODINE, J. H., 1921.—Changes in hydrogen ion concentration and titratable acidity and alkalinity of hay and soil cultures. Biol. Bull. **41**, No. 2

BRENCHLEY, W. F., 1911, 1912, 1913.—The weeds of arable land. Ann. Bot. **25**, 155; **26**, 95; and **27**, 141.

[1] With but few exceptions, references to be found in Clark's (1920) list have been omitted here.

Brown, P. E., and Warner, H. W., 1917.—The production of available phosphate from rock-phosphate by composting with sulphur and manure. Soil Sci., **4**, 269.

Cameron, F. K., and Briggs, L. J., 1901.—Equilibrium between carbonates and bicarbonates in aqueous solution. J. Phys. Chem., **5**, 537.

Chambers, C. O., 1912.—The relation of algae to dissolved oxygen and carbon dioxide, with special reference to carbonates. 23rd Ann. Report, Missouri Bot. Gardens, 171–207.

Clark, W. M., 1920.—The determination of hydrogen ions. Baltimore.

Clarke, F. W., 1916.—Data of Geochemistry. U.S.A. Geol. Survey, Bull. 616.

Cole, S. W., 1920.—Practical physiological chemistry. Cambridge.

Comber, N. M., 1921.—Relation of the hydrogen ion concentration of the soil to plant distribution. *Nature*, Sept. 29th, **108**, 146.

Compton, A., 1921.—Rôle of the reaction of the medium in fixing the optimum temperature of a ferment. Proc. R. Soc., **92B**, 1–5.

Davis, W. A., 1918.—A study of the indigo soils of Bihar. Indigo Publ. No. 1. Imp. Dept. of Agric., India.

Davis, W. A., 1920.—The effect of manuring with superphosphate and sannai on the yield of crops on indigo planters' estates in Bihar. *Loc. cit.*, No. 6.

Dixon, H. H., 1914.—Transpiration and the ascent of sap in plants. London.

Dixon, H. H., and Atkins, W. R. G., 1915.—On the constituents and concentration of the sap in the conducting tracts, and on the circulation of carbohydrates in p ants. Sci. Proc. R. Dub. Soc., **14** (N.S.), 374; and Notes, Bot. School, Trin. Coll., Dub., **2**, 275.

Duchartre, P. E. S., 1859.—Ann. Sc. Nat., Ser. 4, **12**, 269 (Botany). Cited.

Falk, K. G., 1915.—Esterase and lipase from castor beans. Proc. Nat. Acad. Sci., U.S.A., **1**, 136.

Falk, K. G., 1917.—Further experiments on lipolytic actions. J. Biol. Chem., **31**, 97–123.

Falk, K. G., and M'Guire, G., 1921.—The sucrolytic actions of bananas. J. Gen. Physiol., **3**, 595.

Felton, L. D., 1921.—A colorimetric method of determining the hydrogen ion concentration of small amounts of fluid. J. Biol. Chem., **46**, 299.

Fisher, E. A., 1921.—1. Studies on soil reaction. J. Agr. Sci., **11**, 19–65. 2. Relation of the hydrogen ion concentration of the soil to plant distribution. *Nature*, **108**, 306.

Flood, M. G., 1919.—Exudation of water by *Colocasia antiquorum*. Sci. Proc. R. Dub. Soc., **15** (N.S.), 506.

FOOT, F. J., 1865.—On the occurrence of *Cystopteris fragilis*, and of *Digitalis purpurea* on limestone, both near Athlone. Proc. Nat. Hist. Soc., Dublin, **4**, 2.

GILE, P. L., and CARRERO, J. O., 1920.—The cause of lime-induced chlorosis and availability of iron in the soil. J. Agr. Res., **20**, 33.

GRIGORIEV, P., 1916.—The alkalinity and acidity of aqueous soil extracts, Russ. j. agri. exper., **17**, 231.

HAAS, A. R. C., 1920.—Studies on the reaction of plant juice. Soil Sci., **9**, 341–368.

HALL, A. D., 1910.—The soil. London.

HARDY, F., 1921.—A preliminary investigation into the occurrence of different kinds of carbonates in certain soils. J. Agr. Sci., **11**, 1.

HARRIS, F. S., 1920.—Soil alkali: its origin, nature, and treatment. Utah.

HARTWELL, B. L., and PEMBER, F. R., 1918.—The presence of aluminium as a reason for the difference in the effect of so-called acid soil on barley and rye. Soil Sci., **6**, 259.

HARVEY, R. B., 1920.—Hydrogen ion changes in the mosaic disease of tobacco plants and their relation to catalase. J. Biol. Chem., **42**, 397.

HESSELMAN, H., 1917.—Studier över saltpeterbildningen, etc. Meddel. f. Statens Skogsförsöksanstalt. Heft 13–14, s. 297 and 923. Abstracted in J. Ecol., 1919, **7**, 210–213.

HOWARD, A., and HOWARD, G. L. C., 1920.—Some aspects of the indigo industry in Bihar. Pt. I. Mem. Dept. Agric., India, **11**, No. 1, 5.

HOF, A. C., 1909.—Iodo-eosin as a test for free alkali in dried-up plant tissues. Biochem. J., **4**, 175.

JAMESON, A. P., and ATKINS, W. R. G., 1921.—On the physiology of the silk-worm. Biochem. J., **15**, 209.

JOFFE, J. S., 1920.—The influence of soil reaction on the growth of alfalfa. Soil Sci., **10**, 301.

KAPPEN, H., 1920.—The form of acidity in soils and its meaning in plant physiology. Landw. Versuchsst., **96**, 277–307.

KEEN, B. A., 1921.—The physical investigation of soil. Science Progress, **15**, 574.

KOBER, P. A., 1917.—An improved nephelometer-colorimeter. J. Biol. Chem., **29**, 155.

LEE, W. T., 1907.—U. S. Geol. Survey, Bull. 315, p. 485.

LIPMAN, J. G., M'LEAN, H. C., and LINT, H. C., 1916.—Sulfur oxidation in soils, and its effect on the availability of mineral phosphates. Soil Sci., **2**, 499.

LIPMAN, J. G., 1919.—Adjusting the soil reaction to the crop. Soil Sci., **7**, 181.

LIPMAN, J. G., BLAIR, A. W., MARTIN, W. H., and BECKWITH, C. S., 1921.—Inoculated sulfur as a plant-food solvent. Soil Sci., **11**, 87.

MACDOUGAL, D. T., 1920.—Hydration and growth. Carnegie Inst. of Washington, Publ. 297.

MACINTIRE, W. H., GRAY, F. J., and SHAW, W. E. M., 1921.—The non-biological oxidation of elemental sulfur in quartz media. Soil Sci., **11**, 249.

M'CALL, A. G., and HAAG, J. R., 1920.—The hydrogen ion concentration of certain three salt nutrient solutions for plants. Soil Sci., **9**, 481.

M'CALL, A. G., 1921.—The relation of the hydrogen ion content of nutrient solutions to growth and chlorosis of wheat plants. Soil Sci., **12**, 69.

MASON, T. G., 1920.—On the inhibition of invertase in the sap of *Galanthus nivalis*. Sci. Proc. R. Dub. Soc., **16** (N.S.), No. 7; and Notes, Bot. Sch., Trin. Coll., Dub., **3**, 105.

MATHEWS, A. P., 1909.—The spontaneous oxidation of the sugars. J. Biol. Chem., **6**, 3–20.

MATHEWS, A. P., and WALKER, S.—The spontaneous oxidation of cystein. *Loc. cit.*, 21–28.

MEACHAM, M. R., HOPFIELD, J. H. H., and ACREE, S. F., 1920.—Preliminary note on the use of some mixed buffer materials for regulating the hydrogen ion concentrations of culture media and of standard buffer solutions. J. Bact., **5**, 491.

MEDALIA, L. S., 1920.—Color standards for the colorimetric measurement of hydrogen ion concentration. J. Bact., **5**, 441.

MENDEL, L. B., and BLOOD, A. F., 1910.—Some peculiarities of the proteolytic activity of papain. J. Biol. Chem., **8**, 177–213.

MIRASOL, J. J., 1920.—Aluminium as a factor in soil acidity. Soil Sci., **10**, 153–217.

MIYAKE, K., 1916.—The toxic acid of soluble aluminium salts upon the growth of the rice plant. J. Biol. Chem., **25**, 23.

MOORE, B., PRIDEAUX, E. B. R., and HERDMAN, G. A., 1915.—Studies of certain photosynthetic phenomena in sea-water. Trans. Liverpool Biol. Soc., **29**, 233–264.

MOORE, B., 1920.—The cause of the exquisite sensitivity of living cells to changes in hydrogen- and hydroxyl-ion concentrations. J. Physiol. (Proc. Physiol. Soc.), **53**, p. lvii.

MUSSET, M., 1865.—Compt. rend., **61**, 683. Cited.

NATHANSOHN, A., 1907.—Ber. u. d. Verhandl. d. k. Sachs. Gesellsch. d. Wissensch. zu Leipzig-math-nat. Klasse. Bd. **59**. Cited.

NEF, J. U., 1911.—Dissoziationsvorgänge in der Zuckergruppe. Liebig's Ann., **403**, 204–383.

ODÉN, S., 1921.—The application of physico-chemico methods to the study of humus. Trans. Faraday Soc. Meeting, May 31st, on Soil.

OSTERHOUT, W. J. V., and HAAS, A. R. C., 1918.—On the dynamics of photosynthesis. J. Gen. Physiol., **1**, 1–16.

PRAEGER, R. L., 1921.—The genus Sedum. J. R. Hort. Soc., **46**, 1–308.

ROAF, H. E., 1920.—Graphic conversion of Sörensen's pH (– log H) into concentrations of hydrogen ions. J. Physiol., **54**, p. xvii.

RUSSELL, E. J., 1912.—Soil conditions and plant growth. London.

SALISBURY, E. J., 1920.—The significance of the calcicolous habit. J. Ecol., **8**, 202.

SCHMIDT, C. L. A., and HOAGLAND, D. R., 1919.—Table of P_H H^+ and OH^- values corresponding to electromotive forces determined in hydrogen electrode measurements, with a bibliography. Univ. of California, Publ. in Physiology, **5**, No. 4, 23–69.

SHARP, L. T., and HOAGLAND, D. R., 1919.—Notes on recent work concerning acid soils. Soil Sci., **7**, 197.

SHEDD, O. M., 1919.—Effect of oxidation of sulfur in soil on the solubility of rock-phosphate and on nitrification. J. Agr. Res., **18**, 329.

SKEATS, E. M., 1902.—Univ. Texas Mineral Survey. Bull. No. 2. Cited.

SKENE, M., 1915.—The acidity of Sphagnum and its relation to chalk and mineral salts. Ann. Bot., **29**, 65–87.

SWANSON, C. O., LATSHAW, W. L., and TAGUE, E. L., 1921.—Relation of the calcium content of some Kansas soils to the soil reaction as determined by electrometric titration. J. Agr. Res., **20**, 855.

TOTTINGHAM, W. E., and HART, E. B., 1921.—Sulfur and sulfur composts in relation to plant nutrition. Soil Sci., **11**, 49.

TURPIN, H. W., 1920.—The carbon dioxide of the soil air. Cornell Univ. Agr. Expt. Sta. Mem. 32.

USSHER, W. A. E., 1907.—The geology of the country around Plymouth and Liskeard. Mem. Geol. Survey.

VAN ALSTINE, E., 1920.—The determination of hydrogen ion concentration by the colorimetric method. Soil Sci., **10**, 467.

WALKER, J., and KAY, S. A., 1912.—The acidity and alkalinity of natural waters. J. Soc. Chem. Ind., **31**, 1013.

WELLS, R. C., 1915.—The solubility of calcite in water in contact with the atmosphere and its variation with temperature. J. Wash. Acad. Sci., **5**, 617.

WHERRY, E. T., 1916.—A chemical study of the habitat of the walking fern, *Camptosorus rhizophyllus* (L) Link. J. Wash. Acad. Sci., **6**, 672–679.

„ 1918.—The reactions of soils supporting the growth of certain native orchids. *Loc. cit.*, **8**, 393–396.

„ 1919.—The statement of acidity and alkalinity with special reference to soils. *Loc. cit.*, **9**, 305–309.

„ 1920.—Determining soil acidity and alkalinity by indicators in the field. *Loc. cit.*, **10**, 217–223.

„ 1920.—Observations on the soil acidity of Ericaceae and associated plants in the Middle Atlantic States. Proc. Acad. Nat. Sci., Philadelphia, **72**, 84–112.

„ 1920.—Correlation between vegetation and soil acidity in Southern New Jersey. *Loc. cit.*, **72**, 113–119.

„ 1920.—Plant distribution around salt marshes in relation to soil acidity. Ecology, **1**, 42–48.

„ 1920.—Soil acidity and a field method for its measurement. Ecology, **1**, 160–173.

„ 1920.—The soil reactions of certain rock ferns. Amer. Fern. J., Pt. I & II, **10**, 15 and 45.

„ 1920.—Soil tests of Ericaceae and other reaction-sensitive families in Northern Vermont and New Hampshire. Rhodora, J. New Eng. Bot. Club, **22**, 33.

ZOLLER, H. F., 1921.—Casein viscosity studies. J. Gen. Physiol., **3**, 635.

XXXIII.

PHOTOSYNTHESIS AND THE ELECTRONIC THEORY (II).

By HENRY H. DIXON, Sc.D., F.R.S.,
University Professor of Botany in Trinity College, Dublin;
AND
NIGEL G. BALL, MA.,
Assistant to the University Professor of Botany, Trinity College, Dublin.

[Read December 20, 1921. Published February 2, 1922.]

It has been pointed out that the energy required in photosynthesis to form carbohydrates from carbon dioxide and water is derived from the light energy rendered available by the displacement of electrons of the chlorophyll molecule. This view is necessitated by the fact that the wave frequencies absorbed by chlorophyll are those which are effective in photosynthesis (1).

Previous experiments have shown that the number of electrons ejected from the molecules of chlorophyll when illuminated by visible light could not carry anything like sufficient energy to make the formation of the observed quantities of carbohydrates possible external to the chlorophyll. Hence it would seem to follow that the necessary electrons are not ejected from, but are merely displaced in the chlorophyll molecule (1). This displacement may be supposed to render some atomic group in the chlorophyll molecule reactive leading to the fixation of carbon dioxide, to the separation of a carbohydrate from its complex molecule, and to reconstitution of the original from the residue and water, with the evolution of oxygen.

This paper contains an account of some experiments carried out with a view to testing further the photo-electric properties of chlorophyll by means of a photographic method.

According to the generally received view, the latent image of the photographic film is formed by the displacement of electrons on exposure to light. The development of the plate reveals the ionization so caused, and converts the latent image into the photographic image. A strong confirmation of this view is to be found in the observation that the latent image may be formed at a temperature so low that no chemical action is possible (2).

An extension of this theory explains the action of sensitizers. Light of wave frequencies, which are unable to form a latent image in the untreated

film, may be rendered effective by tinging the film with a dye which absorbs those wave frequencies. Here the electrons of the dye-molecules displaced by the absorbed light are regarded as the cause of the ionization necessary to form the photographic image on development.

This view would receive additional support if it could be shown that sensitizers are effective at temperatures at which chemical actions do not take place.

Sensitization at Low Temperatures.

To test this point experiments were made on the following lines. Ilford process plates, which are comparatively insensitive to red light, were cut up into a number of small plates, 1 cm. × 2 cm. Half of each of these was sensitized by immersing it for a few seconds in a solution of "Sensitol Red" (1 : 76,000), as supplied by Ilford, Ltd., and made up according to the maker's directions. After the plates had dried, one was attached to a disc of blackened cardboard (the "back"), 2 cm. in diameter, by a rubber band passing lengthwise across the plate, and at right angles to the line of junction between the sensitized and untreated portions of the plate. The back was then fixed at one end of a blackened cardboard tube, about 30 cm. long, the other end of which was closed with a transparent Wratten light filter, No. 29, transmitting only red light of wave-lengths greater than 615$\mu\mu$. The cardboard tube, which formed a long camera, was set upright in a flask-shaped Dewar vessel, so that the lower end containing the back was submerged in the liquid air held in the vessel. The upper end projected from the neck. The liquid air in the vessel, entering by perforations in the lower part of the tubular camera, covered and completely submerged the plate. The Dewar vessel and camera were set directly under a 100-watt "gas-filled" lamp. The plate was about 40 cm. distant from the lamp. All these arrangements and operations were performed in total darkness. Exposure was made by switching on the lamp. Exposure for one hour under these conditions produced a latent image, which on development with rodinal showed only a slight silver deposit on the untreated portion of the plate. A clear strip—the shadow of the rubber band—without silver deposit crossed this portion. The rest of the plate which had been sensitized showed out dark in contrast; but it, too, was crossed by the clear shadow.

This experiment, which was frequently repeated, shows conclusively that the sensitizer is effective at the temperature of liquid air (−185° C), and hence supports the electronic theory of sensitization. There is, however, a marked loss of sensitiveness at this temperature, for a control plate with precisely the same arrangements, save that the flask did not contain liquid

air and was at room temperature, showed a stronger deposit of silver after thirty seconds' exposure than did the experimental plate after an hour's exposure. Furthermore, it should be noticed that the effectiveness of the sensitizer is considerably reduced at this temperature, for it is always observed that the contrast between the sensitized and untreated portions is much greater on the film exposed at room temperature than on that submerged under liquid air during exposure.

These experiments show that the photographic film may be used as a means of testing the electronic disturbances caused by light in substances distributed through it. It will be noticed that the effects on the film will be summative, and, therefore, given time, even when the number of electrons displaced per second is small, sensible effects may be produced.

Sensitization by Chlorophyll.

It is well known that chlorophyll, like many dyes, acts as a sensitizer to the photographic film, and the fact that its presence renders the photographic film sensitive to those wave frequencies which it absorbs is strong evidence that, in its case also, exposure to light leads to the displacement of electrons. This conclusion would be rendered almost certain if sensitization by chlorophyll could be shown to be effective at the temperature of liquid air.

Nearly all experimenters on photographic sensitization by chlorophyll comment on its uncertainty. The uncertainty of its action is particularly noticeable when it is used in conjunction with gelatine photographic films. As gelatine plates are so manageable, and give such excellent results with other pigments, it seemed worth while to carry out some experiments to try and ascertain the conditions necessary for their sensitization.

Experiments on the Sensitization of Gelatine Films.

Untreated photographic gelatine films were immersed in alcoholic extract of fresh leaves, alcoholic extract of dry leaf-powder, and alcoholic extract of leaves killed by exposure to steam. None of these preparations of crystalloidal chlorophyll gave certain results.

Similarly untreated plates immersed in or smeared with colloidal chlorophyll were equally uncertain.

The addition of different quantities of ammonia to the sensitizing bath—although some good plates were obtained by this method—did not render sensitization certain. Neither did the acidulation of the bath produce better results.

An effort to change the sign of the colloidal chlorophyll by the addition of calcium sulphate did not secure sensitization.

The reverse process was also tried, namely, to change the sign of the gelatine of the film, but it was found that plates soaked in a solution of barium nitrate were no more easily sensitized than untreated ones.

The influence of the physiological condition of the chlorophyll itself was also tested; thus extracts from leaves recently exposed to light were compared with those made from leaves previously shielded from light. No difference was observed. In the same way extracts made and stored in darkness showed themselves no different from those which had been exposed to the light.

These preliminary experiments afforded no hopes that certain results could be readily obtained with gelatine photographic plates. Consequently it was decided to abandon gelatine films and to carry out the experiments with collodion emulsion in spite of its inconveniences.

It was noticed, however, that in the occasional cases in which satisfactory sensitization was obtained with gelatine plates it was effective, not only at room temperature, but was also observed on portions of the same plates exposed under liquid air.

Experiments on the Sensitization of Collodion Films.

The first experiments which we made with collodion were carried out with an emulsion made up in the laboratory. The plates, which were coated and allowed to dry for about five minutes, were then immersed in a leaf-extract. Subsequent drying lasted half an hour, and then the plate was exposed to light, as previously described in the case of the gelatine films, at room temperature for sixty to eighty seconds. Sensitization was easily obtained both with ordinary dry-leaf extract and with an alcoholic solution of chlorophyll *a* and *b* purified from the accompanying yellow pigments and fats.

With exposures for thirty minutes and sixty minutes under liquid air the result was inconclusive, as the whole plate appeared somewhat fogged, and no clear strip corresponding to the shadow of the band was observed.

Similar collodion plates sensitized with "Sensitol Red" in a similar manner gave good results, both for room temperature and when submerged in liquid air. At room temperature (as in the case of gelatine plates) the effectiveness of the sensitizer, as shown by the contrast between the sensitized and untreated portions of the plates, was much greater.

Further experiments were carried out, using a sample of collodion prepared and supplied by Messrs. Johnson & Sons, Finsbury, London. The coated plate after five minutes' drying was partially immersed in leaf-extract. After this immersion it was again allowed to dry for ten to fifteen minutes. Exposure to light for three hours was then made while the plate was submerged in

liquid air. On development satisfactory sensitization was apparent. The development with rodinal lasted in each case about three minutes.

These results, which were repeated several times, establish the fact that sensitization with chlorophyll of photographic films, whether of collodion or of gelatine, is effective at the temperature of liquid air. Inasmuch as in these experiments it is only light of visible wave-lengths, viz., $\lambda = 615\mu\mu - 725\mu\mu$, which is absorbed by the chlorophyll, and produces the ionization forming the latent image, it follows that this light displaces electrons of the chlorophyll. Previous experimental work has shown that visible light does not expel electrons from chlorophyll.[1] Hence it follows that the energy absorbed from visible light is wholly or in part used in the displacement of electrons within the chlorophyll molecule, making some atomic group or groups within it reactive. In photosynthesis, we may suppose that these groups react with the raw supplies, viz., carbon dioxide and water.

We might imagine the process to take place as follows :—

$$\underset{\text{(chlorophyll } a\text{)}}{C_{55}H_{72}O_5N_4Mg} + CO_2 = \underset{\text{(chlorophyll } b\text{)}}{C_{55}H_{70}O_6N_4Mg} + CH_2O$$

$$\underset{\text{(chlorophyll } b\text{)}}{C_{55}H_{70}O_6N_4Mg} + H_2O = \underset{\text{(chlorophyll } a\text{).}}{C_{55}H_{72}O_5N_4Mg} + O_2$$

Such a scheme would account for the constant proportions of chlorophyll *a* and *b*. The fact that the formation of formaldehyde cannot be detected when chlorophyll in presence of carbon dioxide alone is exposed *in vitro* (3) may be explained by assuming, with Siegfried (6) and Willstätter (8), that the fixation of carbon dioxide with a protein is first necessary, and that the decomposition of the carbamino-acid or carbaminate is effected by the reactive group in the chlorophyll produced by the absorption of light. The formation of chlorophyll *b* and formaldehyde from chlorophyll *a* and this loosely combined carbon dioxide would probably take place instantaneously on exposure to light. A momentary inflow of carbon dioxide to make up the loss of carbamino-acid or carbaminate might be expected; but the subsequent rate of absorption would depend on the rapidity with which the carbamino-acid was broken down by the available chlorophyll *a*, and would gradually rise to a steady state as the normal proportion of chlorophyll *a* to chlorophyll *b* was

[1] It is of interest here to record an unpublished observation of J. Joly and J. H. J. Poole. These investigators, using an extremely sensitive method of investigation—the tilted leaf electroscope—found that another sensitizer, viz., Sensitol Red, like chlorophyll, is practically non-photoelectric under the action of visible light.

re-established. Such a rise in the rate of photosynthesis during illumination, as determined by the fixation of carbon dioxide, has been observed by Osterhout and Haas (5), and explained by them, "by assuming that sunlight decomposes a substance whose products catalyze photosynthesis or enter directly into the reaction."

It may be assumed that these reactions only take place when the leaf is exposed to light, and that their velocity under normal conditions is dependent on the intensity of illumination. The velocity of the first reaction would, however, be increased by increased illumination only so long as carbon dioxide is in excess, whereas the velocity of the second reaction would not be directly influenced by this factor. It is probable, therefore, that in strong sunlight the balance between the two reactions would be disturbed, tending to an increased proportion of chlorophyll *a*.

The following figures, deduced from the means of Willstätters' results (7), seem to bear out this view :—

Proportion of Chlorophyll a to Chlorophyll b.

Name of plant.	Sun leaves.	Shade leaves.
Sambucus nigra,	2·745	2·030
Aesculus hippocastanum,	2·687	2·268
Platanus acerifolia,	3·370	3·100
Fagus silvatica,	3·075	2·875

In the plant exposed to light in absence of carbon dioxide one would expect that chlorophyll *b* would be very much diminished in amount, if not altogether absent. This does not seem to have been tested by experiment.

The authors wish to express their indebtedness to Mr. T. G. Mason, M.A., B.SC., for some preliminary experimental work on the sensitization of gelatine photographic plates, and also to the Royal Dublin Society for supplies of liquid air, which made the experiments at low temperature possible.

During the earlier stages of the work Mr. Ball was in receipt of a Maintenance Grant from the Department of Scientific and Industrial Research.

REFERENCES.

1. DIXON, H. H., and POOLE, H. H.—Photosynthesis and the Electronic Theory. Proc. R.D.S., vol. xvi (N.S.), No. 5, March, 1920.

2. JOLY, J.—Latent Image. Presidential Address to the Photographic Convention of the United Kingdom, July, 1905. *Nature*, vol. lxxii, p. 308.

3. JÖRGENSEN, I., and KIDD, F.—Some Photochemical Experiments with pure Chlorophyll. Proc. Roy. Soc. B., vol. clxxxix, 1917, pp. 342–361.

4. JÖRGENSEN, I., and STYLES, W.—Carbon Assimilation. New Phytologist Reprint, No. 10, 1917.

5. OSTERHOUT, W. J. V., and HAAS, A. R. C.—Dynamical Aspects of Photosynthesis. Proc. National Academy of Sciences, vol. iv, pp. 85–91, April, 1918.

6. SIEGFRIED, M.—Ueber die Bindung von Kohlensäure durch Amphotere Amidokörper. Zeitschrift für Physiologische Chemie. Bd. 44, 1905, p. 85.

7. WILLSTÄTTER, R., and STOLL, A.—Untersuchungen über Chlorophyll. Berlin, 1915.

8. WILLSTATTER, R., and STOLL, A.—Untersuchungen über die Assimilation der Kohlensäure. Ber. d. Deutsch. Chem. Gesellsch. Sept. 1915.

XXXIV.

THE BIONOMICS OF THE CONIDIA[1] OF *PHYTOPHTHORA INFESTANS* (MONT.) DE BARY.

By PAUL A. MURPHY, B.A., A.R.C.Sc.I.,
Assistant in the Seeds and Plant Disease Division, Department of Agriculture and Technical Instruction for Ireland.

[Read December 20, 1921. Published February 2, 1922.]

In previous papers (18, 19)[2] accounts were given of field experiments in Canada and in Ireland bearing on the occurrence of blight in potato tubers, particularly after digging. It was shown that the bulk of the infection in the case of potatoes which develop blight in storage is contracted when the tubers are being dug. Proof was given that contact of the tubers with partially blighted foliage results in serious rot in storage. Evidence was also presented to show that soil contaminated with conidia shed from the leaves continues capable of inducing blight in freshly dug tubers which are brought into contact with it for a period of at least ten days, and probably longer. It was mentioned that the part played by contaminated soil was fully established by the results of laboratory investigations. The present paper deals with this phase of the subject.

I.—Vitality of Conidia.

Vitality of conidia in soil out-of-doors.—As it is generally accepted, following de Bary (4), Jensen (11), Hecke (10), McAlpine (14), Jones, Giddings and Lutman (12), and Melhus (17), that the life of the conidia in comparatively dry air is measured in hours rather than days, an attempt was made to determine the effect of keeping the conidia in soil.[3] A preliminary

[1] The word "conidium" is used throughout to describe the asexual reproductive body which behaves sometimes as a conidium and sometimes as a zoosporangium.

[2] The numbers in brackets refer to the list of literature cited at the end of the paper.

[3] Jensen (11) found by experimental methods that conidia may survive in soil for five days. Apart from this experiment, no other reference to the subject has been found in the literature, with the exception of certain observations of de Bary (3) on the vitality of conidia in soil which had carried a blighted crop; of Melhus (16) on the behaviour of conidia produced on the surface of infected tubers in the soil; and of Peters, as reported by Karsten (13), on the continued vitality of infecting material placed on the soil. Although the matter was not followed up, the conclusions reached point in the same direction as our own results, particularly in the last two cases. See also Appel (1).

experiment indicated that by so doing the spores remained viable and capable of infecting potatoes for several days.

On September 15th the following experiment was begun. Finely sifted potting loam, in a comparatively dry condition, was dusted on the lower surface of potato leaves which carried a strong growth of conidiophores of *Phytophthora infestans*, and was then brushed off again with a fine brush. In this way a quantity of soil, weighing about 400 grams, was so copiously contaminated that it was easy to find conidia in any fraction when examined microscopically. The soil so prepared was divided into four parts, each of which was placed in the form of a little mound on the surface of similar loam which filled a flower pot. Four similar pots were prepared in which were placed like quantities of sifted potting loam which had not been contaminated. All the pots were placed on a roof exposed to the weather but shaded from the sun for part of the day. The length of time the conidia survived was determined by inoculating a little of the soil from the mounds into shallow wounds in healthy tubers.[1]

Using this method, it was found that the fungus remained capable of infecting tubers for twenty-one days, but not for twenty-eight days. The first inoculations on the unwounded surface were failures; thenceforth they were made in wounds, with the result that on the 13th day, 8 out of 8 inoculations were successful; on the 19th day, 4 out of 8; on the 20th day, 4 out of 6; on the 21st day, 3 out of 6; on the 28th day, 0 out of 8. Throughout the experiment no disease developed in any of the thirty-two wounds in which was placed soil similarly exposed but not contaminated with conidia.

This experiment was conducted during the driest portion of the year, from September 15th to October 6th, 1920. Notwithstanding their exposed position the conidia survived this. Torrential rain occurred on October 2nd

[1] Examinations were first made microscopically, but it was found that searching for viable conidia in soil, and then testing their germination, was a time-consuming operation. Reliance was, therefore, placed on infection experiments on potato tubers with adequate controls. At first whole tubers were used, the inoculum being introduced into wounds, while similar potatoes from the same lot were wounded but not inoculated. The bulk of the work, however, was carried out on potato slices, and a portion from every tuber used was left untreated. In all the critical experiments an "adjacent surface control" was allowed to every inoculated slice, that is, the two surfaces which were in contact before a tuber was cut were used as a check on each other, one being inoculated and the other not. It may be stated that of the 232 control pieces used throughout all the experiments to be described, only four failed to remain sound, and none developed blight. In no single case did non-contaminated soil produce blight, and it was always used for comparison with contaminated soil. In all the work, with the exception of some of the preliminary experiments (in which the typical appearance of blight rot was deemed sufficient), infection was not admitted until the parasite was recovered from the infected material.

and 3rd, but it was not until three days after this that the last successful infection was found.

Vitality of conidia in clay, sand and salts.—As the contaminated soil which was the first to fail to produce the disease in the last experiment was in a pot which had been taken indoors during the previous few days, the effect was tried of mixing conidia with a quick-drying medium, such as silver sand, in comparison with clay. As it had been claimed[1] that the conidia were capable of living over winter in a mixture of calcium sulphate and calcium carbonate, this medium was also tested. The clay, sand, and salts were kept in Petri dishes.

The results are shown in Table I. The clay mixture produced blight up to twenty-one days after being made up, the sand mixture up to seven days, and the salts (although they were perfectly dry throughout) up to twelve days. No disease developed from any of the forty-two control inoculations in which clay, sand and salts respectively were used.

TABLE I.—Length of life of conidia in clay, silver sand, and salts mixture in room.

	Successful infections after						
	3 days.	4 days.	5 days.	6 days.	7 days.	12 days.	21 days.
Clay, .	6/6	6/6	6/6	6/6	6/6	6/6	5/6
Sand, .	5/6	6/6	6/6	4/6	1/6	0/6	—
Salts, .	6/6	6/6	6/6	6/6	6/6	1/6	—
Controls,	0/6	0/6	0/6	0/6	0/6	0/6	0/6

(In the case of the numbers expressed as fractions, the denominator represents the number of inoculations and the numerator the number of infections secured.)

In this experiment the sand and clay were slightly moist, and as nearly uniformly so to begin with as possible. The sand became noticeably drier during the course of time, and to this fact, possibly, the early death of the fungus is to be attributed. On the other hand, the salts mixture was air-dry to begin with, and it was left in this condition. The surprising length of time which the fungus survived in it appears to be attributable to the fact

[1] Griffiths, Chemical News, vol. 53, 1886, p. 255. This experiment was repeated under the conditions given (in a dry incubator at 35° C.) but with an entirely different result from that claimed.

that the conidia became coated with the medium, a feature which is also found in examining contaminated clay.

Effect of moisture on the vitality of conidia in soil.—In order to determine the effect of excessive moisture and lack of moisture on conidia in the same kind of soil, three ordinary flower pots were filled with moderately dry potting loam. In the surface of the soil in each pot there was sunk a small paper cylinder, the diameter and depth of which were each about 2·5 cm., and the cylinders were then filled with loam as uniformly contaminated as possible with conidia from potato leaves. Four days afterwards, when the soil had settled, one pot was watered copiously with a fine rose until water ran freely from the bottom; another pot was soaked by standing it in water until the soil was absolutely saturated; the third was left unwatered. Thereafter, until the end of the experiment, no more water was added. The three pots were kept in a cool room, the central portions, including the paper cylinders, being covered with Petri dish lids. Throughout the greater part of the forty days of the experiment the pots maintained different degrees of moisture, the soaked pot being water-logged, and by far the wettest, the watered pot intermediate, and the unwatered pot driest. The contaminated soil was tested periodically by transferring portions of it to slices of potato tubers.

The result was, as may be seen from Table II, that the dry and moist soils reproduced the disease for forty days, while that in the water-logged pot did so (though rather irregularly towards the end) for twenty-six days. Beyond this period, in both cases, the result was uncertain, for while some rot followed after the later inoculations, the blight fungus was not recovered. This feature was noted rather regularly towards the end of several experiments. It may be that the small amount of *P. infestans* which remained viable set up a weak rot and was soon swamped by saprophytes; or perhaps, as has been noted several times, old and apparently dead mycelium and conidia have a toxic action on potato tissue, setting up small local lesions which sometimes provide a foothold for other micro-organisms. Thirty-six control inoculations, using similarly treated but non-contaminated soil, gave negative results. It is, therefore, clear that, contrary to the views accepted since the time of de Bary, *P. infestans* may remain capable of attacking potatoes in the soil, even when the latter is water-logged, for comparatively long periods. The most favourable conditions for survival are found under the more or less dry conditions reproduced in the "dry" and "moist" soils. At the close of the experiment there was little difference in the amount of moisture present in these two pots.

TABLE II.—Length of life of conidia in dry, moist and water-logged loam.

Days from beginning of experiment.	Successful infections.		
	Dry soil.	Moist soil.	Water-logged soil.
1	4/4	4/4	4/4
3	4/4	4/4	4/4
5	4/4	4/4	4/4
8	4/4	4/4	4/4
10	4/4	4/4	3/4
13	4/4	4/4	3/4
16	4/4	4/4	4/4
19	4/4	4/4	1/4
22	4/4	4/4	0/4
26	4/4	4/4	1/4
30	4/4	4/4	0/4
33	4/4	4/4	0/4
40	3/4	3/4	0/4

(In the case of the numbers expressed as fractions, the denominator represents the number of inoculations and the numerator the number of infections secured.)

Effect of temperature and moisture on the vitality of conidia in soil.—A further experiment was carried out with similarly contaminated loam which was kept in small glass sample-tubes tightly stopped with cotton-wool. These tubes were placed three together in larger tubes, also stopped with cotton-wool, and put standing mouths downwards. This procedure was intended to arrest drying up without unduly cutting off the air supply. The tubes were stored under various conditions, as shown in Table III. The soil was dry and friable to begin with. It was left in this condition in some cases, and in others was made moist without being saturated. At intervals of a few days inoculations were made on potato slices, with the following results:—

TABLE III.—Length of life of conidia in loam kept in glass tubes.

Nos. and places of storage.	Original condition of soil.	No. of days after which successful infections were made.												
		3	6	9	12	14	17	20	23	26	30	34	37	44
1. Out-of-doors, Nov. 6–Dec. 20, . .	Dry	4/4	4/4	4/4	—*	—	—	—	—	—	—	—	—	—
2. Do., ...	Moist	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4
3. Unheated room, ...	Dry	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	0/4	0/4	0/4	—
4. Do., ...	Moist	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	3/4	2/4	2/4	?	?
5. Heated room, ...	Dry	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	0/4	0/4	0/4	—	—
6. Do., ...	Moist	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	2/4	1/4	2/4	—
7. Incubator at 20° C. Saturated atmosphere,	Dry	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4
8. Incubator at 25° C. Dry atmosphere, .	Dry	4/4	4/4	4/4	0/4	0/4	0/4	0/4	0/4	—	—	—	—	—
9. Incubator at 30° C. Saturated atmosphere,	Dry	4/4	4/4	4/4	4/4	4/4	4/4	4/4	3/4	1/4	0/4	0/4	?	—
Soil and tuber controls, ...	—	0/12	0/12	0/12	—	—	—	—	—	—	—	—	—	—
Tuber controls, ...	—	—	—	—	0/4	0/2	0/3	0/4	0/4	0/4	0/4	0/4	0/4	0/4

* Tube lost.

(In the case of the numbers expressed as fractions, the denominator represents the number of inoculations and the numerator the number of infections secured.)

The longest period during which the fungus was definitely proved to have retained its vitality in soil was forty-four days. It is worthy of note that with two of the tubes (Nos. 2 and 7) infection was just as easy and certain after the lapse of this period as at the beginning. From the same two tubes infection of potato slices was again secured sparingly after sixty-six days, and a still further trace of infection was obtained from No. 2 after seventy-four days, but in none of these cases was *P. infestans* recovered. Pieces of tubers inoculated after the last two intervals from other tubes (sixteen in number) and with similar non-contaminated soil (eight in number) remained sound.

The conditions under which the fungus survives longest are evidently determined more by moisture than by temperature. The longest periods of activity were found in tubes which were either moist to begin with or were kept in a saturated atmosphere, irrespective of the temperature up to and including 20° C. At 30° C. the temperature becomes important in spite of the abundance of moisture. The most fatal conditions are those which combine high temperature and dry soil.

Attempts at direct examination of contaminated soil.—Direct examination of the soil failed to give an explanation of these findings. It was concluded, after careful search, that the fungus did not survive in the form of mycelium. On the other hand, apparently perfect conidia, among others, were still seen in soil to which they had been added more than three weeks previously. The majority of the conidia observed under such conditions appeared, however, to be shrunken but ungerminated. Others were empty, and from the presence of apical openings were concluded to have produced zoospores. No germ tubes were seen. Soil samples such as these, when placed on potato slices, infected them freely, while similar slices not inoculated remained sound, so that in the apparent absence of mycelium it was concluded that the conidia themselves had the power of retaining their vitality for comparatively long periods.

An attempt to prove this by placing fresh conidia on twelve dry cover glasses in a small chamber, the atmosphere of which was saturated with water-vapour, proved only partially successful. Twelve other cover glasses similarly provided with conidia and prepared at the same time were placed in a similar chamber which was kept dry. The period of life was tested by infecting potato slices (with controls) from the fifth day onwards. The conidia from the moist chamber produced disease regularly until the ninth day, but not afterwards. Neither the conidia from the dry chamber nor the controls produced disease on any day. A later experiment in which the moist chamber was closed with a water-seal led to no better result.

Notwithstanding these findings, based as they are on laboratory experiments, there are indications that the life of conidia in air may be longer under more natural conditions. When a vigorous growth of conidiophores is left undisturbed for some time, it is noticeable that the fringe of the growth contains conidia which are few in number and of poor germinative capacity. Further in towards the substratum numerous and more vigorous conidia may be found. It is probable that the air in the centre of such masses of mycelium is more moist than in the fringe, which would account, to some extent, for the result. At the same time the comparatively low vitality of conidia when removed from the conidiophores and kept in a saturated atmosphere would go to show that another factor besides moisture must be involved. It is possible that large masses of mycelium contain an abnormal proportion of carbon dioxide, arising from the hyphae themselves, the substratum, or from bacteria and other contaminations, and that this has an influence on the conidia. The bearing of a reduced oxygen supply on the germination and vitality of the fungus is discussed more fully in later paragraphs.

The view sometimes expressed (3, pp. 38 and 39; 4), however, that that the conidia may live for some weeks in dry air under certain conditions, while in moist soil their existence is shorter owing to germination supervening after the first rain, is in opposition to our results.

An experiment was twice repeated in which columns of sifted loam and silver sand, each 10 cm. deep, were contained in two upright glass tubes, each having a diameter of 2·8 cm. The tubes were open at the top, and had an arrangement to permit of drainage water escaping at the bottom. After the materials in the tubes had settled, a small quantity of contaminated sand was placed on the surface of the loam and sand. Water in measured quantities was then allowed to drip at fixed rates on the contaminated soil in the tubes. The liquid which came through at the bottom was caught on filter papers, which were transferred at regular intervals to the cut surfaces of potato tubers. The experiments were continued for six and five days respectively.

It was found in these experiments that the filter papers became sparingly pathogenic to potato tubers after the passage of water equivalent to a "rainfall" of 40 to 80 cm. or more, and seventeen to forty-one hours after the "rain" began. In the first experiment the sand alone allowed the infecting material to pass through, this occurring after twenty-six and a half hours, and none coming through the loam. In the second test (begun twelve days after the commencement of the first, the same tubes and soils being used as before, but with the addition of fresh contaminated sand) infecting material passed through the loam filter only, and that after seventeen, twenty-four

and forty-one hours. In the light of later experience, the possibility that this material was left in the loam from the previous experiment must not be overlooked. The most significant point was that in three cases out of four in both experiments the originally contaminated surface-soil was more pathogenic to potatoes, even after a period of three to three and a half days, during which water equivalent to 162 cm. (approximately) of "rain" had passed through it, than the drainage water was at any time. The longest period of pathogenicity of the surface-soil under the conditions of continuous "rain" slightly exceeded four and a half days.

II.—Germination of Conidia.

Previous work on the germination of the conidia.—It was conceived that the method by which the conidia germinated might have a bearing on the period of vitality, zoospores being probably more delicate than germ tubes. It was found difficult, however, to deduce from previous work which method might be expected from conidia in the soil.

While Berkeley and Montagne (6) saw the early stages of zoospore formation within the sporangium, and de Payen (20) observed the emptying of the latter, the meaning of the process was unknown to them and to the earlier workers, such as Morren in 1845 (according to Hecke), Schacht (22) and Speerschneider (24), all of whom observed direct germination.[1] It was not until 1860 that the production of zoospores was recorded by de Bary (2) for *P. infestans.* De Bary (3, 4) was not able to find exactly what the conditions determining the two methods of germination were. He was first of the opinion that germ tubes were formed only when the tubers were infected in the ground, but later found that this did not always hold. He regarded zoospore formation as the normal procedure, and suggested that germ tubes were produced only by old conidia of low germinative power. Strong sunlight prevented the formation of zoospores. A third method of germination described by this author (2, 3, 4) for the first time consisted of tube formation followed at once by the production of a secondary conidium. De Bary regarded this as a specific mode of germination, due to partial immersion in water. The secondary conidium had the power of producing zoospores, or a tertiary conidium might result.

Marshall Ward (25), to whom we owe the fullest and most accurate description of the germination process, considered that zoospore formation was favoured by the presence of oxygen in the water and by the absence of

[1] That is, the production of germ tubes. When zoospores result, the germination is said to be indirect.

organic matter, such as jam or dung, and of too strong sunlight. Tube germination followed by the formation of secondary conidia, while depending to some extent on the conidia themselves, was favoured by light, thick sowing, and by any cause which delayed germination. A temperature factor did not seem to be involved.

Hallier (9) found that zoospores were formed only when food was lacking, while germ tubes resulted in nutrient solutions. Smorawski (23) apparently arrived at the same result. This conclusion Hecke (10) also largely supports; but in so far as he found no zoospores produced in solutions containing more than 0·5 per cent. of dry matter, while germ tubes occurred not only in such solutions but were sometimes associated with zoospores in very large numbers in distilled water, he concluded that some peculiarity must lie in the conidia themselves. His conclusion was that very young conidia only ("eben erst gebildete Konidien") can produce zoospores, but that they do so imperfectly (with partial extrusion of the contents) in nutrient solutions. Older conidia never form zoospores. Their germination is poor in distilled water and generally closes with a secondary conidium, while in nutrient solutions they germinate well.

Brefeld's (8) conclusion with regard to zoospore production in the Peronosporaceae (including *P. infestans*) was that thorough wetting favoured this form of germination.

McAlpine (14) found that the first crop of conidia produced zoospores, while the later crops were variable. A short period of drying prevented zoospore formation.

Jones, Giddings and Lutman (12) found that potato juice favoured direct germination and that temperature also affected the process. "At 25° C. more than 50 per cent. of the germinations are by tubes, . . . while at 10° to 20° C. direct germination is exceptional."

According to Melhus (17) the determining factor does not consist of age or any innate peculiarity of the conidia; nor of light, the concentration of the solution, or the amount of oxygen available. On the other hand, temperature is all-important. At 13° C. the maximum of zoospore formation is found, while at about 23° C. this has practically ceased and germ tubes are the rule. It is only in extremely strong nutrient solutions (20 per cent. dextrose) that zoospore formation is replaced by some direct germination.

Fresh experiments on the germination of the conidia.—The results of previous work on the conditions regulating the manner of germination being found so conflicting that no conclusion could be drawn, the problem had to be taken up afresh. Fortunately in the course of this investigation it was

found possible to reproduce conditions in microscopical preparations under which the conidia remained viable and capable of infecting tubers for several weeks. As these conditions resemble in many respects those which occur in soil, it is reasonable to suppose that the explanations which will be given of the phenomena observed in the preparations are also applicable to natural conditions.

This portion of the work also resulted in extending our knowledge of the morphology and biology of the fungus in some important particulars, one of which at least has a bearing on the fate of conidia in the soil.

The findings of Hecke and Melhus were first compared. For this purpose the germination of conidia was tested in the following liquids, which were sterilized before using:—(1) distilled water, (2) tap water, (3) soil extract, prepared by the slow filtration of tap water through four inches of soil, and (4) a 1·5 per cent. solution of glucose in distilled water. To observe the effect of temperature, one set of germination tests in these media was carried out at room temperature (which during these trials never sank below 10° C. at night, or rose above 15° C. during the day, with one exception, when 22° C. was reached), while another set was carried out in a constant temperature incubator at 22°–23° C. Both sets were kept in the dark. Conidia were derived from Petri dish cultures on potato slices, which were infected from pure cultures on oat agar. Every care was taken to use uniform material from the same culture for both sets of tests. The germinations were carried out under cover glasses (except in some special cases), and the slides were kept in moist chambers to prevent drying. It is possible in this way to keep the same preparation under observation for several weeks, and to change the liquid at will without materially disturbing the conidia.

Comparison of the effects of nutrient solutions and temperature on germination.—Our work on the conditions (so far as they were tested) governing the germination of the conidia agrees best with that of Marshall Ward (25). These conditions were found to be very complicated, as would be expected from the results of earlier investigations. The comparison of the effect of the solution and of the temperature was repeated seven times without achieving a constant result, as may be seen in Table IV.

TABLE IV.—Influence of nutrient solutions and of temperature on the germination of conidia.

Date of Experiment.	Distilled water.		Tap water.		Soil extract.		Glucose 1·5 per cent. sol.	
	Germination.	Method.	Germination.	Method.	Germination.	Method.	Germination.	Method.
10°–15° C.								
23. 2. 21	Good	S only	None	—	Good	C (24) S (16)	Good	S (25) C (4)
24. 2. 21	Poor	S ,,	Good	S only	Poor	S only	Good	S only
25. 2. 21	Fair	S ,,	Poor	S ,,	Fair	S ,,	Poor	S ,,
26. 2. 21	Good	S (122) C (2)	Fair	S (20) C (4)	Good	S (91) C (11)	Good	S ,,
28. 2. 21	Good	S only	Good	S only	Good	S (99) C (22)	Good	S ,,
1. 3. 21	Good	S (*n*) C (1)	Good	S ,,	Good	S only	Good	S ,,
2. 3. 21	Good	S (*n*) C (1)	Good	S (*n*) C (1)	Good	S (*n*) C (1)	Fair	S ,,
22°–23° C.								
23. 2. 21	Fair	C only	None	—	Fair	C only	Poor	C only
24. 2. 21	Trace	C ,,	Poor	C only	Good	C ,,	Trace	C ,,
25. 2. 21	Trace	C ,,	Poor	C ,,	Good	C ,,	Trace	C ,,
26. 2. 21	Fair	C ,,	Fair	C ,,	Poor	C ,,	Trace	C ,,
28. 2. 21	Trace	C ,,	Trace	C ,,	Fair	C ,,	None	—
1. 3. 21	Fair	S ,,	Good	S (56) C (17)	Trace	C ,,	Good	S only
2. 3. 21	Fair	C ,,	Trace	C only	Trace	C ,,	Fair	C ,,

S stands for spores functioning as sporangia; C for those functioning as conidia. The figures following in some cases represent the relative numbers counted. The symbol (*n*) represents a very large number.

The following conclusions from this experiment seem to be justified (see Table IV):—

Temperature of 10°–15° *C.*

1. At a temperature of 10°–15° C. the regular method of germination is by means of zoospores. With good material the germination runs from 50 to nearly 100 per cent.

2. Generally speaking, the number of conidia producing germ tubes at this temperature is small, but it may under certain conditions exceed the number producing zoospores.

3. The influence of nutrient solutions on the production of germ tubes was not apparent in the case of the sugar solution used, but there was, perhaps, somewhat more evidence for it in the case of soil extract. A somewhat larger number of abortive (unescaped) zoospores was found in the sugar and soil solutions than elsewhere.

4. There is considerable evidence that the tendency to produce germ tubes at a temperature of 10°–15° C. rests in the conidia themselves (see test of 26. 2. 21 at 10°–15° C.).

Temperature of 22°–23° *C.*

1. At 22°–23° C. the regular method of germination is by means of germ tubes. The germination seldom exceeds 50 per cent., and is generally less.

2. Ordinarily the production of zoospores is rare, but conidia with undifferentiated contents but with the papilla gone, or with contents extruded, or containing abortive zoospores, are frequent.

3. On one occasion (test of 1. 3. 21 at 22°–23° C.) the production of zoospores largely replaced that of germ tubes, indicating either a peculiarity on the part of the conidia then used, or that temperature does not act directly on germination, but influences it through some factor which it generally (but not always) calls into play.

4. The influence of nutrient solutions was not marked, but soil extract seemed to favour tube formation.

The tests carried out at 22°–23° C. gave percentages of germination which were uniformly much lower than those found at the lower temperature. It was evident that the conidia were near the limits of their development. It is believed that the results recorded have little, if any, bearing on the conditions under which blight epidemics occur.

Effect of age on the method of germination.—Some conidia germinate by means of germ tubes under conditions which cannot be explained by reference

to aëration, presence of other organisms, temperature or nutrient solutions. From a consideration of the frequently different behaviour of two conidia lying close together under apparently similar conditions, one is forced, along with most previous workers, to conclude that different potentialities must reside in the bodies themselves. This was one of de Bary's views, and his suggestion that germ tubes are the result of age and lack of vigour has been endorsed by many of his successors without any convincing proof being adduced to support it.

Evidence in support of this view can, however, now be given. Conidia were tested from an eight-months'-old pure culture on oat agar. The aërial portion of the mycelium at least appeared dead, and it must have been a considerable time since any conidia had been produced. There were, however, some perfect conidia present among a multitude of dead ones. The former germinated in sterile water at 10°–15° C., somewhat slowly but strongly, and entirely by means of germ tubes. A peculiar feature of their germination was that the tube or tubes (often up to five) occasionally sprang from all parts of the wall, although generally they came from the normal place beside the papilla. These conidia were also peculiar in possessing a yellowish oil drop and an unusually prominent papilla, just like secondary conidia. It is conceivable that this is the outward sign of their having gone through some maturation process.

Similarly with conidia placed in water, whether primary or secondary, if they do not germinate within about twenty-four hours they seem practically incapable of producing zoospores. Here again in most cases (always, indeed, with secondary conidia) we get the same signs of an inward change. Furthermore, when such conidia lie long in water (three weeks), the germ tube may arise from any part of the wall.

An attempt, however, to distinguish between conidia from a comparatively fresh growth which germinate by means of tubes soon after being placed in water at 10°–15° C., and those with a similar origin which produce zoospores, resulted in failure. Many conidia apparently similar in all respects were marked, some of which afterwards germinated in one way and some in the other. It was observed that only the hyaline conidia germinated at all. Those which were at all dark-coloured or slightly plasmolysed failed to grow.

III.—Effect of Lack of Oxygen on Development of Conidia.

Germination delayed or prevented by lack of oxygen.—During the course of these tests it was noted several times that the presence of bacteria or yeasts

arrested germination. It soon became possible to distinguish between conidia which were incapable of development and those the development of which was merely delayed. The least sign of plasmolysis is a sure index of death, but before this appears a peculiar darkening and granulation of the protoplasm indicate, according to our experience, that the conidium is incapable of development.

It was found that some slides (particularly those kept at 23° C.) became badly contaminated with bacteria and yeasts, and that under such conditions germination was confined to a zone about 4 mm. wide near the edge of the cover glass. Within this ring a negligible amount of germination, or none whatever, was to be seen. If, however, the slide was very carefully and repeatedly irrigated with its appropriate solution, the process being watched under the microscope and extreme caution being exercised not to disturb the conidia, it was found that the germination of a large number of the central conidia could be brought about. Three hours after irrigation the result was sometimes apparent, but sometimes it took twenty-four hours. While traces of indirect germination have been seen under these circumstances, germ tubes are almost the invariable rule, irrespective of the temperature or of the nature of the solution.

This experience was repeated many times, at first involuntarily and afterwards of set purpose. A test in tap water at 23° C., begun on 2. 3. 21, became contaminated with bacteria after twenty-four hours, and then showed only 18 conidia with germ tubes out of 198 conidia examined. Most of these were near the edge, there being very few in the centre. After irrigation and a further period of twenty-four hours, the number of conidia with germ tubes was 112 out of 271 examined. Another slide containing soil-extract and belonging to the same series also became contaminated and showed four conidia with germ tubes out of a large number examined after twenty-four hours. On irrigation the number rose to 34 in 89.

That it was the presence of bacteria which prevented germination was proved repeatedly by setting up some tests under practically sterile conditions, while in others bacteria were introduced. The former (at 10°–15° C.) showed numberless zoospores after about three hours, and after twenty-four hours the majority of the conidia had germinated in this way all over the slide. Where bacteria had been introduced perfect zoospore formation was found only in a narrow zone near the edge of the cover glass, while there might be attempts at zoospore production elsewhere. If now fresh water were copiously introduced a great access of germination resulted in most cases, germ tubes only being produced. Control slides to which no water was added showed no change, or at most an entirely negligible amount of tube

production. Corresponding results were secured with preparations kept at 22°–23° C.

This experiment was repeated using motile protozoa along with conidia, which makes a beautiful preparation. The protozoa after twenty-four hours are confined to a narrow zone near the edge of the cover glass, in which they swim about freely. In this area, and extending somewhat within it, good germination is to be found, whilst still further in there may be no germination whatever. Motile bacteria exhibit the same zonation to some extent, the outer ones only being in motion. Penicillium spores have also been used with somewhat similar results. In this case the rapid development of *P. infestans* allows some zoospores to be produced, but a great many become abortive.

These results, it is believed, are to be explained by the fact that the competing organisms use up the oxygen in the water and replace it with carbon dioxide faster than the conidia can utilize it for the purpose of germinating. There may be a question of other excretion products having an inhibitory effect, but judging from the variety of organisms used this is hardly likely. The same effect has been produced by sealing round the edge of the cover glass with melted paraffin. Furthermore, the conidia themselves, if the slide is very thickly seeded, bring about the same result. When germination is general around the outside of a clump, there is often none at all in the centre. Conidia scattered among the hyphae of living mycelium of *P. infestans* frequently behave similarly.

Formation of secondary conidia.—Secondary conidia, as originally described by de Bary (2, 3, 4), are the immediate products of previously existing conidia, being borne on the germ tubes of the latter. The name "secondary conidia" is not a particularly good one, for conidia morphologically and physiologically identical are produced by hyphae which fructify in water, and identical but smaller bodies may be formed by zoospores, as will be shown. The term is used in a somewhat loose sense in this paper for the new conidia which sometimes follow after the germination both of conidia and of zoospores. Such secondary conidia seem to owe the characteristic appearance which is common to them and to conidia produced by submerged hyphae to the fact that they are formed in water, on which account a word like "hydro-conidia," which would include all three, might be preferable.

The conditions under which germination is arrested but subsequently takes place are favourable for the production of secondary conidia. These have been very common on our slides. When germ tubes are formed in the presence of bacteria, or when bacteria are introduced after their formation, secondary conidia will always be met with within a zone from about 2 to 4 mm.

wide inside the edge of the cover glass. In preparations sealed with paraffin they are found only at the extreme edge. Their formation is constant, and is the result of specific conditions. At a certain degree of oxygen concentration which just permits germination, or when the oxygen supply is gradually cut off after germination has taken place, secondary conidia are produced. Bacteria are the most reliable agents for bringing about this state of affairs, as may be seen by introducing them into one of two uniform preparations. At a still lower oxygen concentration ungerminated conidia are eventually (and sometimes rapidly) killed; but there is evidence derived from sealed preparations to show that germ tubes continue living for several weeks, and even increase in length, with a still scantier oxygen supply. This is possibly connected with the greater amount of exposed surface, which facilitates respiration. For some reason which has not been established, secondary conidia appear to be materially more resistant than are conidia formed in air to the adverse influences they meet with in water in competition with other organisms; and they are, therefore, an important adaptation to certain aquatic conditions.

The formation of secondary conidia has been mentioned by most workers since their discovery by de Bary (2, 3, 4), but certain new observations may be recorded. The conidia which give rise to them rapidly lose all their contents, which pass in their entirety into the smaller and less symmetrical secondary bodies. If the primary conidium has two germ tubes a secondary conidium may be produced by each. The hypha joining the primary and secondary conidia is of variable length, or may exceptionally be lacking, the second conidium being an asymmetrical prolongation of the first. The germ tubes generally grow in the direction of the greater oxygen concentration. Frequently the new conidia are found in the free water surrounding the cover glass, being borne on very long tubes springing from conidia lying further in. These hyphae grow straight to the margin, perhaps after a preliminary turn. The secondary conidia have thinner walls and more rounded and prominent papillae than the original ones, and they always contain one (or occasionally two) yellowish oil-drops. Even when lying in the free water around the cover glass they may remain ungerminated but viable for about three weeks. Frequently, however, they germinate sooner, and they may produce zoospores if the conditions are favourable; but germ tubes are the general rule, frequently followed by a third conidium, as de Bary and others have described. These have frequently been seen, and on several occasions even what may be called a quaternary conidium, four conidia in a chain, each somewhat smaller than the preceding one. It is conceivable that repeated germination and conidium formation may result in

a further lease of life, analogous to the survival of mycelium under circumstances which are fatal to fresh conidia.

Zoospores and the production of "secondary" conidia.—When conidia germinate by the production of zoospores, the latter, in the presence of sufficient oxygen, settle down after a longer or shorter period of activity, develop a cell wall and immediately put out a germ tube. There is no doubt that oxygen in sufficient quantity favours this whole proceeding. When oxygen is lacking the germination is often abortive, even at favourable temperatures. The zoospores may be more or less differentiated, but none may emerge; or some or all may escape, but they do not swim far from the sporangium and soon come to rest. Under such conditions it frequently happens that no cell wall is formed, and the zoospore dies and dissolves. That it is lack of oxygen which limits the activity of the spore in these respects is shown by the presence of naked and otherwise abortive zoospores among masses of Phytophthora mycelium. The coming to rest of large numbers of zoospores in a cluster, which is sometimes seen under these conditions, is explainable on similar grounds. The chance coming of one or more into a zone of low oxygen concentration puts an end to their activity, and the accentuation of the dearth of oxygen which their presence causes entraps other zoospores which happen to swim into the locality, and so the process goes on.

When the zoospores germinate under favourable conditions, the germ tube is of such enormously greater capacity than the original spore, and continues increasing in length (even though not completely filled with protoplasm), or at least remains alive and vigorous-looking so long, that a saprophytic existence is at once suggested (7). However this may be, it is certain that the zoospore germ tubes can live for one week in water, for they have been directly observed to do so for that period. The statement of de Bary (3) that they cannot live longer than twenty-four hours under such conditions is not correct. A single week, however, is not the limit of their existence. They, too, have the faculty of producing "secondary" conidia, an observation which does not seem to have been recorded previously. The conditions are the same as for conidium production by conidia—namely, a reduction in the amount of oxygen. The phenomenon was first observed in a sealed preparation where conidia had discharged zoospores surrounding an air bubble. Six days afterwards there was an attempt at conidium formation by a zoospore germ tube, but it did not come to fruition. Subsequently in a preparation originally set up under sterile conditions and kept for five days at 10°–15°C., in which there had been abundant zoospore formation, the

almost universal production of "secondary" conidia by the zoospores situated near the edge of the cover glass was observed. The slide had become contaminated with bacteria. These new conidia were borne on the ends of long spirally twisted germ tubes. They contained all the protoplasm, even of the branches, where such were present. In form they exactly resembled those produced by conidia, being asymmetrical, and provided with prominent papillae and yellow oil-drops, but they were much smaller, although having a considerably greater volume than the original zoospore. In a companion preparation, which was not contaminated, the zoospores did not produce "secondary" conidia.

It is evident that the faculty by which germinating zoospores may prolong their existence under unfavourable conditions by developing directly into conidia must be of significance in the life-history of the fungus. The general and rapid production of zoospores by conidia at relatively low temperatures and in subdued light is evidently an adaptation to facilitate leaf infection during the heavy night dews of late summer and autumn. It is probable that under these conditions failure to bring about infection in the night or morning hours results in the death of the spores, at least in dry weather. In the case of conidia which fall to the ground, even though they function as sporangia, it is clear, in the light of our present knowledge, that the resulting zoospores are not nearly so delicate as to lose their vitality (unless they succeed in infecting a potato tuber) a short time after the first shower of rain which brings about their liberation. The conidia produced by zoospores have probably a measure of the same power of resistance as is possessed by other secondary conidia, so that instead of a single body capable of living for days or weeks and then infecting the plant under favourable conditions, the eventual sources of infection are multiplied manifold. The extent to which this multiplication can go was not determined, but "secondary" conidia, the product of zoospores, were seen which were empty and open at the apex as though they, in turn, had again produced swarm spores.

Relation of results obtained in microscopical preparations to soil conditions.— The germination of secondary conidia was observed twenty-four days after the original conidia were placed in water, and the germ tubes of the former were still living after thirty-four days. Potatoes were infected many times with conidia which had been fifteen to sixteen days in water (during which time they had germinated, and in some cases produced further conidia), the entry of the germ tubes into thin slices of tubers being observed under the microscope. The hyphae grew between the cells, and fine convoluted branches were sent into the cells and between the starch grains. Where secondary conidia were not formed, the germ tubes were found to be an

important aid in prolonging life. While original ungerminated conidia produced in air were not observed to be viable after a period of five to seven days in water, germ tubes and secondary conidia are much more resistant.

The low oxygen concentration in which the slow development and continued vitality of the fungus were directly observed for comparatively long periods in microscopical preparations is believed to offer an explanation of the similar resistance exhibited in the soil. It is reasonable to suppose that all the conditions reproduced artificially must be realized in the soil, as well probably as many others.

According to Russell and Appleyard (21), there are two distinct atmospheres in the soil—the "free air" and the "dissolved air." The former does not differ in principle from atmospheric air, but it contains a larger and fluctuating amount of carbon dioxide (about 0·25 per cent.), and is almost saturated with water vapour. The dissolved air contains practically no oxygen, and is made up of carbon dioxide and nitrogen in varying proportions. It is shown that the oxygen requirements of the soil are always in excess of the supply, and thus the two bodies of air, though existing in close relationship to each other, retain their individual characteristics. Temperature, cultivation, manuring, and rainfall in particular, however, affect the soil atmospheres. A heavy fall of rain, which is nearly saturated with oxygen, partially renews the dissolved air. This, as evidenced by the subsequent rise in the CO_2 content, allows the development of aërobic organisms. Among the latter the conidia of *P. infestans* must find in the fluctuations of the dissolved atmosphere the conditions which enable them to survive for the long periods noted in the experiments with contaminated soil, and to germinate when conditions are made more favourable by the accession of water rich in oxygen.

There is another feature in the fluctuation in the amount of carbon dioxide in the soil which may influence the course of the blight fungus. The channel along which the parasite makes its way from the tubers to the leaves in spring is not known with sufficient certainty. The stem of the potato has been proved in a few cases to be penetrable by the mycelium, but the possibility of the transference taking place through the soil has hardly been studied. This is the more curious since the downward course of the parasite in the autumn from the leaves to the tubers is admittedly through the soil. It may be significant that the time at which the parasite finds its way downwards coincides with the minimum CO_2 content of the "free" soil atmosphere, while in the spring, when the course is upwards, the CO_2 content is at its maximum. The results of an experiment to test the capacity of the blight fungus to penetrate the soil were so suggestive that further attention is being devoted to the subject.

SUMMARY.

The conidia of *Phytophthora infestans* when mingled with soil and kept out of doors may remain viable and capable of infecting potato tubers for a period of between three and four weeks.

The vitality of the conidia is retained longer in loam than in a quick-drying medium like silver sand.

In experiments indoors conidia survive longer in comparatively dry to somewhat moist soil than in very wet soil, the respective periods being forty and twenty-six days.

Temperatures up to and including 20° C. have no ill effect on the conidia as long as sufficient moisture is present. At 30° C. the temperature is not too high for the conidia to survive for twenty-six days if the atmosphere is saturated. The most fatal conditions are those which combine lack of moisture and high temperature.

Under the most favourable conditions indoors, conidia in soil infected tubers just as freely after forty-four days as when fresh.

Conidia when placed in soil survive without the production of obvious mycelium. The attempt to preserve ungerminated conidia in air saturated with water-vapour met with comparative failure, nine days being the apparent limit of existence.

Initially contaminated surface soil, after the continuous passage through it of large quantities of water, may remain pathogenic to potatoes for a period exceeding four and a half days, and generally infects potatoes more freely than the water after passing through 10 cm. of soil does at any time during the period.

As having a possible bearing on the resistance of conidia in soil, their method of germination was re-examined in the light of previous work, which is summarized.

At a temperature of 10°–15° C. the conidia germinate freely, and generally produce zoospores. A small number of germ tubes may, and often do, result; while exceptionally they may be in the majority. Nutrient solutions, such as soil filtrate and 1·5 per cent. glucose in water, are without material influence in favouring tube production; but such solutions hinder somewhat the perfect formation of zoospores. Abundant oxygen and the absence of competing organisms favour zoospore production.

The tendency to produce germ tubes at low temperatures seems to be innate in the conidia themselves. Conidia which have remained for some time ungerminated in air and those which have had their germination in water delayed for any reason seem capable only of giving rise to germ tubes.

At a temperature of 22°–23° C. the limit of development of the conidia is approached. The germination is comparatively poor and germ tubes are the general rule. While abortive sporangia are frequent, the production of perfect zoospores is rare. Exceptionally, zoospores may largely take the place of germ tubes. The reason for this is obscure, but is believed to lie in the conidia themselves.

Secondary conidia and old but still viable conidia from air resemble each other in possessing a more prominent papilla than usual, and a large yellowish oil-drop. These appear to be signs of their having undergone some maturation process which increases their resistance. Both as a rule produce germ tubes, generally more than one, which may spring from any part of the wall.

The germination of conidia in water is delayed by lack of oxygen, and may subsequently be brought about by the timely supplying of fresh water. Suspension of germination has been effected by means of bacteria, protozoa, Penicillium spores, thick seeding of conidia, and by ringing cover glasses with paraffin; and a good measure of germination has thereafter been effected by adding fresh water.

Secondary conidia, so called, are constantly produced at a certain degree of oxygen concentration which just permits germination, or when the oxygen supply is cut off after germination has taken place. In microscopical preparations they are confined to a zone near the edge of the cover glass, within which germ tubes may live and grow without having the power of forming new conidia. Ungerminated conidia from air are rapidly killed under the latter conditions.

Secondary conidia appear to be conidia adapted to an aquatic existence, similar bodies being produced by hyphae which fructify in water. They are more resistant than primary conidia, and this resistance is increased by the faculty they possess of successive germination followed by fresh conidium formation, which may be repeated four times. They may also produce zoospores under favourable conditions.

Zoospore germ tubes may also develop at once into "secondary" conidia when the oxygen supply is reduced after germination has taken place. Zoospore germ tubes may live for at least seven days in water, and their life is further lengthened when new conidia result. The secondary bodies so formed may apparently again produce zoospores.

The limit of existence of fresh ungerminated conidia in competition with other organisms in water is about five to seven days, and the period may be much shorter. After a germ tube is produced the resistance of the fungus is increased. Potato tubers have frequently been infected with such conidia fifteen to sixteen days after being placed in water. The germination of

secondary conidia has been observed twenty-four days after the original conidium was placed in water, and the resulting germ tubes lived at least ten days longer.

The conditions under which the continued existence of conidia, zoospores and germ tubes in water was directly observed for periods exceeding, in some cases, thirty days are believed to represent the conditions under which existence is possible for comparatively long periods in the soil.

LITERATURE CITED.

1. Appel, O.—Beiträge zur Kenntnis der Kartoffelpflanze und ihrer Krankheiten I. Arb. K. biol. Anstalt Land.- und Forstwirtsch., Band 5, Heft 7, pp. 377–435, 1907.

2. Bary, A. de.—Sur la formation de zoospores chez quelques champignons. Premier mémoire. Ann. des Sciences Nat., 4 Ser. Zool., T. 13, pp. 236–251, 1860.

3. Bary, A. de.—Die gegenwärtig herrschende Kartoffelkrankheit, ihre Ursache und ihre Verhütung. 75 pp. Leipzig, 1861.

4. Bary, A. de.—Recherches sur le développement de quelques champignons parasites. Ann. des Sciences Nat., 4 Ser. Bot., T. 20, pp. 5–148, 1863.

5. Bary, A. de.—Researches into the Nature of the Potato-fungus *Phytophthora infestans.* Jour. R. Agric. Soc. England, Ser. 2, vol. 12, pp. 239–269, 1876. Also in Journal of Bot., N.S., vol. 5, pp. 105–126 and 149–154, 1876.

6. Berkeley, Rev. M. J.—Observations, Botanical and Physiological, on the Potato Murrain. Jour. Hort. Soc. London, vol. 1, pp. 9–34, 1846. Includes some work of Montagne's.

7. Brefeld, O.—Botanische Untersuchungen über Hefenpilze. Heft. 5. Leipzig, 1883 (see p. 8 *et seq.*).

8. Brefeld, O.—Untersuchungen aus dem Gesamtgebiete der Mykologie. Band 14. Münster i. W., 1908 (see p. 115 *et seq.*).

9. Hallier, E.—Neue Untersuchung der durch *Peronospora infestans* Casp. herforgerufenen Krankheit der Kartoffeln. Zeitschr. f. Parasitenkunde, Band 4, Heft 3, p. 263. Ref. Just's Bot. Jahresber., Jahrg. 3, p. 999, 1875.

10. HECKE, L.—Untersuchungen über *Phytophthora infestans* De By. als Ursache der Kartoffelkrankheit. Jour. f. Landw., Band 46, Heft 1, pp. 71–74; Heft 2, pp. 97–142, 1898.

11. JENSEN, J. L.—Moyens de combattre et de détruire le Peronospora de la pomme de terre. Mém. Soc. Nat. d'Agric. de France, T. 131, 130 pp., 1887.

12. JONES, L. R., N. J. GIDDINGS and B. F. LUTMAN.—Investigations of the Potato Fungus *Phytophthora infestans.* U.S. Dept. of Agric., Bureau of Plant Industry, Bull. 245, 93 pp., 1912. Also issued as Bull. 168, Vermont Agr. Exp. Station.

13. KARSTEN, H.—Zweiter Bericht über die von den landwirtschaftlichen Akademieen und Versuchstationen . . . in den Jahren 1864 und 1865 ausgeführten Untersuchungen über die Kartoffelkrankheit . . . Ann. d. Landw., Band 49, pp. 104–122, 1867.

14. MCALPINE, D.—Some points of practical importance in connexion with the Life-history stages of *Phytophthora infestans* (Mont.) de Bary. Ann. Mycol., vol. 8, No. 2, pp. 156–166, 1910.

15. MELHUS, I. E.—The perennial mycelium of *Phytophthora infestans.* Centbl. f. Bakt., &c., Abt. 2, 39 Band, pp. 482–488, 1913.

16. MELHUS, I. E.—Hibernation of *Phytophthora infestans* of the Irish potato. Journ. Agric. Research, vol. 5, No. 2, pp. 71–102, 1915.

17. MELHUS, I. E.—Germination and Infection with the Fungus of the Late Blight of Potato (*Phytophthora infestans*). Agric. Exp. Stn., University of Wisconsin, Research Bull. 37, 64 pp., 1915.

18. MURPHY, P. A.—Investigation of Potato Diseases. Dom. of Canada Dept. of Agric., Bull. No. 44 (Second Series), 86 pp., 1921.

19. MURPHY, P. A.—The Sources of Infection of Potato Tubers with the Blight Fungus, *Phytophthora infestans.* Sc. Proc. R. Dublin Soc., vol. 16 (N.S.), pp. 353–368, 1921.

20. PAYEN, A. DE.—Développement et réactions du *Botrytis infestans* de la pomme de terre. Compt. Rend. hebd. de l'Acad. d. Sc., T. 25, pp. 696–699, 1846.

21. RUSSELL, E. J., and A. APPLEYARD.—The Atmosphere of the Soil: its Composition and the Causes of Variation. Journ. Agric. Science, vol. 7, Pt. 1, 48 pp., 1915.

22. Schacht, H.—Bericht an das Königliche Landes-Oeconomie-Collegium über die Kartoffelpflanze und deren Krankheiten. 29 pp. Berlin, [1854].

23. Smorawski, J.—Zur Entwickelungsgeschichte der *Phytophthora infestans* (Montagne) De By. Inaugural-Dissertation Univ. Erlangen. 17 pp., 1890. Also in Landw. Jahrb., Bd. 19, 1890.

24. Speerschneider, J.—Dass das Faulen der Kartoffelknollen bei der sogennanten Kartoffelkrankheit durch die angestreuten und keimenden Sporen des Blattpilzes (*Peronospora devastatrix*) verursacht wird, durch Experimente bewiesen. Flora, N. Reihe 15 Jahrg., No. 6, pp. 81–87, 1857. Similar paper in Bot. Zeit., 15 Jahrg., No. 8, pp. 121–125, 1857.

25. Ward, H. M.—Illustrations of the Structure and Life-history of *Phytophthora infestans*, the fungus causing the Potato Disease. Quart. Journ. Microscop. Sc., vol. 27, N.S., pp. 413–425, 1887.

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